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PATHOLOGIC CHANGES IN GOUTY ARTHRITIS

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IN SPITE of the relative frequency with which an accurate clinical diagnosis of gouty arthritis can be made,[1] there is seldom an opportunity to study the changes in bones and joints removed at operation, and there are few reports of such changes in the literature. In the present case, amputation performed for another condition afforded the opportunity to study several joints, and the pathologic observations will be reported in this paper.

REPORT OF A CASE

A 64 year old shipping clerk was admitted to the University of Chicago Clinics, Aug. 2, 1945, because of inability to walk during the preceding four and a half months.

He had been told that when he was about 9 months old pain and swelling developed in his right knee. After these symptoms had subsided, he began to walk normally, but when he was 5 years old the knee "began to draw up." The deformity was treated by hamstring tenotomies, which resulted in a knee that was straight but stiff. Gradually the deformity returned, and for at least fifty years the knee had been fixed in 110 degree flexion. Every few months he would have a bout of pain in the knee which lasted a few days and subsided spontaneously. In spite of these difficulties, he had been able to work regularly.

Seven months before his admission he had an attack of dizziness without unconsciousness, after which he noted difficulty with his speech and weakness of his right arm and leg. Because of this he went to a hospital where he was treated by rest in bed for a "stroke." While he was there he was awakened one night by pain in his right great toe. The blood uric acid level the next day was 6.5 mg. per hundred cubic centimeters, with all other laboratory tests normal, and the diagnosis of gout was made. Treatment with colchicum was instituted, the attack subsided, and the patient went home fairly well.

Two and a half months later he fell heavily on his right side, and from then on he had so much pain in his knee that he was unable to walk. If he sat still and made no attempt to bear weight he had no pain except at night after he was in bed.

The patient had also noted dyspnea and substernal pain on exertion. There was a history of tuberculosis in his father, brother and sister.

Physical examination revealed an obese elderly white man with little facial expression. His chest was normal. His blood pressure was 150 systolic and

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1. McCracken, J. P.; Owen, P. S., and Pratt, J. H.: J. A. M. A. **131**:367, 1946.

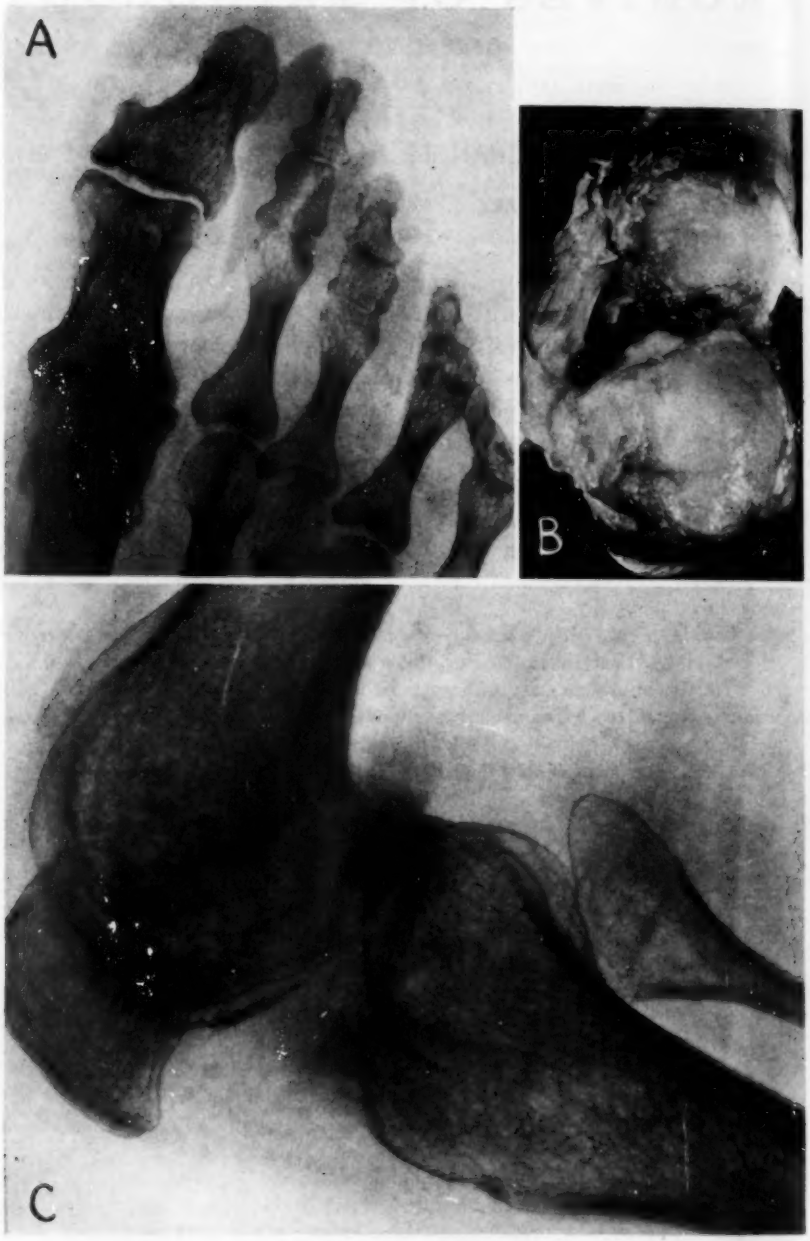

Fig. 1.—In *A* the narrowed cartilage spaces, the sclerosis of the subchondral bone and the irregular marginal lipping of the joints of the big toe are roentgenographic evidence of degenerative arthritis.

B, marked degeneration and deformity of the joint surfaces of the right knee and atrophy of disuse of all bones. Note the concentric atrophy of the fibula, which suggests that the disability started in childhood.

C, photograph of the metatarsophalangeal joint of the big toe showing deposits of urates. Note that there is no bony ankylosis.

90 diastolic. His right arm was weak and stiff, and the reflexes were hyperactive. There was marked atrophy of the entire right lower extremity. There was a 20 degree flexion deformity of the hip and moderate fixed external rotation. The knee, in which there was barely perceptible motion, was flexed 110 degrees,

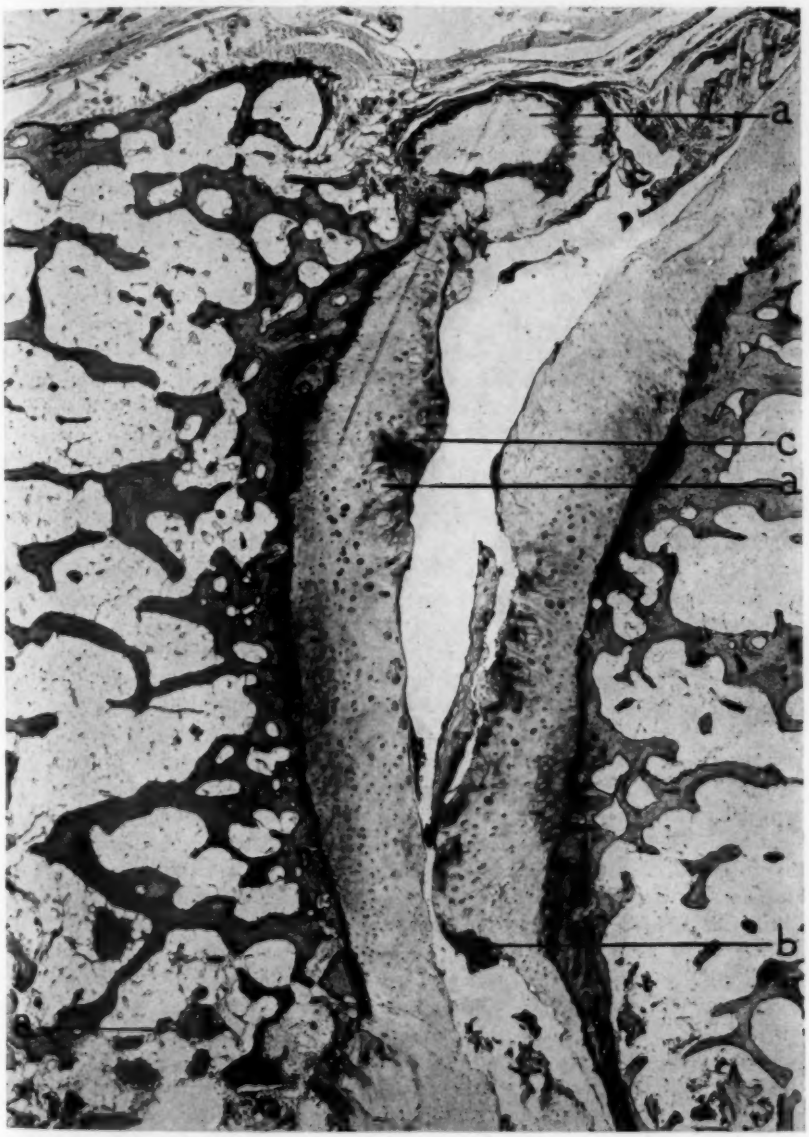

Fig. 2.—Photomicrograph of a sagittal section of the metatarsophalangeal joint showing urates deposited in the synovial membrane, the marrow, and the cartilage (*a*); a thick vascular synovial membrane growing over the surface as a pannus (*b*); a greatly degenerated articular cartilage, which in places is necrotic and beginning to calcify (*c*).

was swollen and felt warm. The right great toe was ankylosed at the metatarsophalangeal joint.

The results of laboratory examinations were all within normal limits except for the concentration of blood uric acid, which was 6.8 mg. per hundred cubic centimeters. The roentgenogram of the chest was normal, and the electrocardiogram showed no pathognomonic changes. The roentgenogram of the foot showed moderate degenerative changes of the joints of the right great toe but nothing characteristic of gout (fig. 1 *A*). The roentgenogram of the knee showed definite evidence of degenerative changes and deformities compatible with old destructive arthritis (fig. 1 *B*). The cartilage space was narrowed, the subchondral cortex irregular, and there was advanced osteophyte formation.

Because of the patient's age and the fact that there was no possibility of restoring function to the knee joint, a low thigh amputation was performed. The patient had an uneventful postoperative course and two months later was walking with a prosthesis and crutches.

On the first and second postoperative days the blood uric acid levels were 9.18 and 9.40 mg. per hundred cubic centimeters. Treatment was begun with colchicine, $\frac{1}{125}$ grain (0.5 mg.) daily for two weeks. At this time medication was stopped because of anorexia, and one month later the uric acid level was 8.68 mg. Although the patient was without symptoms, his diet was adjusted, and five months later the blood uric acid level was 4.26 mg. per hundred cubic centimeters.

On gross examination of the amputated limb, pathologic changes could be noted in all joints except the ankle. The knee joint was the most severely damaged. There was advanced destruction of the articular cartilages, and all surfaces were firmly bound together by abundant dense fibrous tissue. There was no bony ankylosis, but the shape of the articulating surfaces had become adapted to the position of extreme flexion so that even when they were freed the knee could not be extended. In a few spots, particularly on the femoral condyles, flaky white deposits were evident. When these were scraped off and examined under the microscope, they were seen to be composed of the needle-like crystals characteristic of the urates. A similar appearance was noted in the joints of the great toe which had been painful (fig. 1 *C*). The small joints of the foot, which had been symptom free, showed more advanced involvement. Where the deposits were scraped off there were revealed deep erosions of the articular cartilage. The synovial membrane in places was thickened and contained minute nodules.

A sagittal section through the motionless interphalangeal joint of the great toe revealed extensive changes (fig. 2). Although a relatively thick layer of articular cartilage persisted, none of it was normal. It stained poorly, it was fibrillated, and the lacunae often contained large numbers of cells. Near the surface there were clumps of colorless amorphous material, around which cartilage cells were flattened, and about some of them, where there had been necrosis of the cartilage, calcification was present. On other portions of the surface erosion of the cartilage was being effected by a vascular type of fibrous pannus. This evidently arose from the synovial membrane, which was much thickened (fig. 3 *A*). The blood vessels were engorged, and there were many chronic inflammatory cells both in foci and scattered diffusely throughout. All through this tissue were many small and large deposits of the white amorphous material, about which lymphocytes and giant cells were gathered in a thick layer. At its juncture with the articular cartilage the proliferating synovial membrane was not only growing over the joint surface but also beneath the cartilage, which it was destroying from below.

The subchondral bone and fatty marrow were relatively normal except in a few places where there appeared small deposits of the amorphous material (fig. 2). Under higher magnification these appeared to be situated in the marrow (fig. 3*B*). The center of each deposit was more dense than the periphery, and

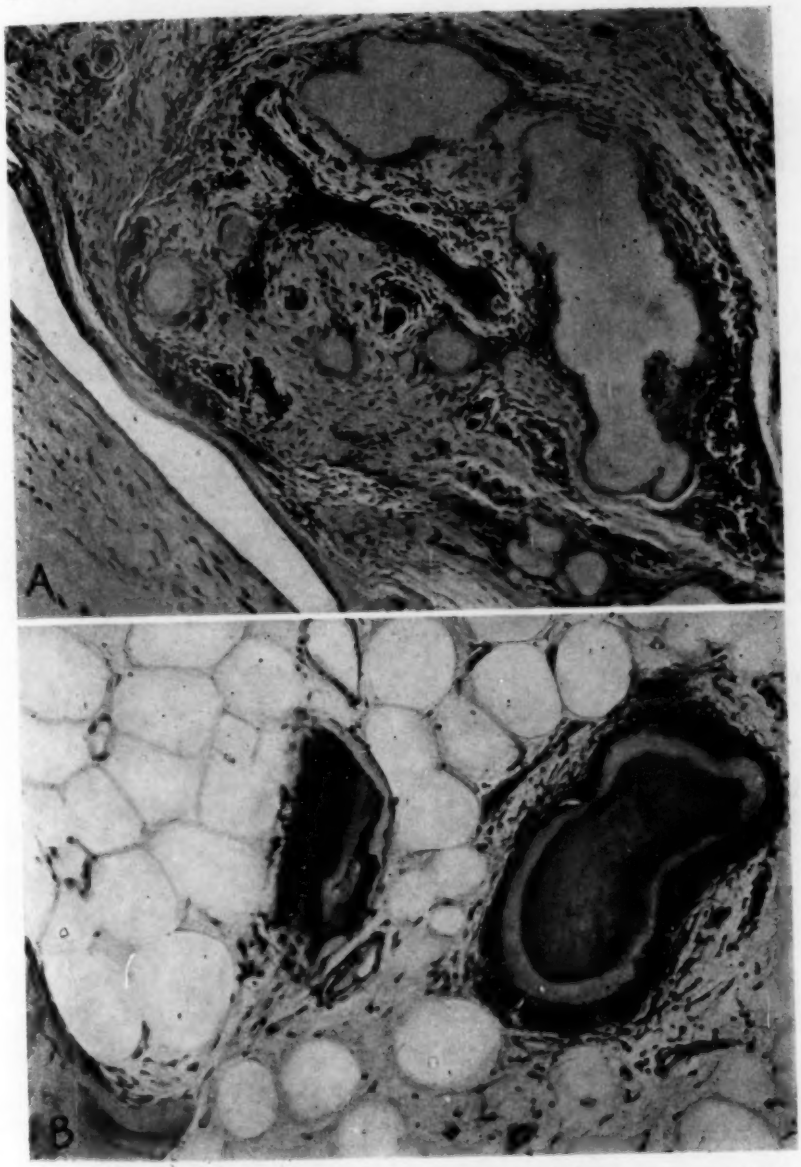

Fig. 3.—*A*, synovial membrane, much thickened, with many engorged blood vessels and nodular deposits of urates.

B, deposit in marrow (see fig. 2). Note the intense but limited inflammatory reaction and the great numbers of multinucleated foreign body giant cells.

each one was surrounded by a narrow zone of intense inflammatory reaction. This shell of vascular connective tissue contained some round cells and so many large multinucleated foreign body giant cells that they formed almost a continuous layer.

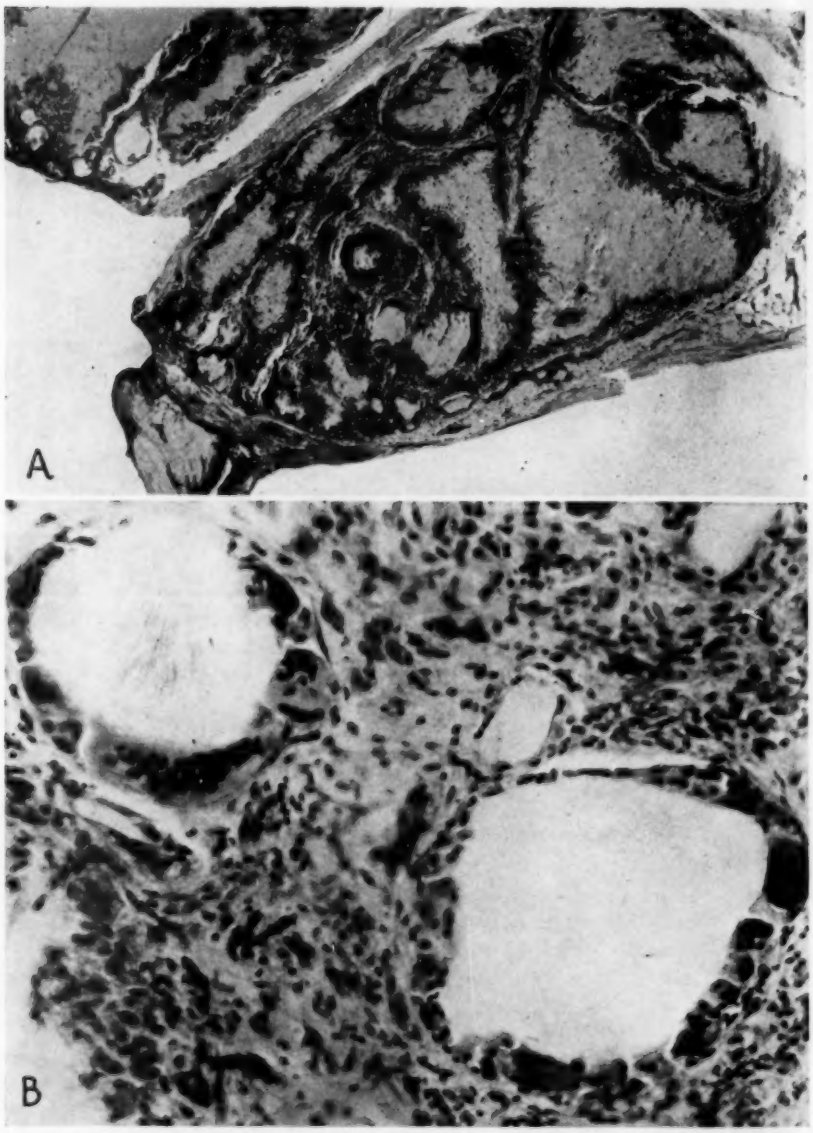

Fig. 4.—*A*, photomicrograph of material removed from a gouty tumor of an olecranon bursa of a patient with classic gout. Note the similarity to figure 3 *A*, with well demarcated nodules separated by fibrous connective tissue.

B, high power photomicrograph of the same lesion with rings of multinucleated giant cells surrounding nodules.

The assumption that the amorphous material surrounded by the foreign body reaction represented deposits of urates is supported by figure 4 *A* and *B*. These photomicrographs show changes identical with those illustrated in the preceding figures. The section was taken from a huge gouty deposit in an olecranon bursa of a patient who had classic gout. Smears of the fresh material showed urate crystals.

COMMENT

Routine fixation of material destroys the characteristic urate crystals of gouty deposits and leaves only the amorphous masses seen in the photomicrographs. For specific demonstration of the crystals in tissue, special technics must be employed.[2] However, the diagnosis can easily be confirmed by microscopic examination of a fragment of the deposit. Also, even after routine treatment, the pathologic changes are unmistakable.

Occasionally the deposit shows two definite concentric layers of crystals as noted in figure 3 *B* and less well in figure 4 *B*. Some workers have analyzed these nodules and found that the peripheral ring is usually composed of sodium urate and the central portion of cholesterol.[3] This separation probably becomes more evident as the lesion grows older, but even in the less well differentiated lesions cholesterol is usually present.[4]

The deposits are seen in articular cartilages, marrow, synovial membranes, joint capsules, bursae, ligaments and tendons.[5] Whenever they occur they provoke an intense inflammatory reaction characterized by many large multinucleated foreign body giant cells which form a ring around the deposits. The synovial membrane of an involved joint thickens and proliferates to form a pannus on the articulating surfaces and also attacks the cartilage from below.[6] In time there is a secondary response to the chronic irritation. The bony margins of the involved joints produce osteophytes, the subchondral bone becomes sclerotic, and the chronic changes of osteoarthritis are added to the acute changes caused by chemical irritation.[7]

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2. de Galantha, E.: Am. J. Clin. Path. **5**:165, 1935.
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4. Bunim, J. J.: Arch. Path. **29**:700, 1940.
5. Musser, J. H.: Am. J. M. Sc. **201**:125, 1941.
6. Henke, F., and Lubarsch, O.: Handbuch der speziellen pathologischen Anatomie und Histologie, Berlin, Julius Springer, 1937, vol. 9, pp. 319-340.
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BRAIN REPAIR

I. Phospholipid-Splitting Enzymes of Brain Phagocytes

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THE MORPHOLOGIC changes following mechanical puncture of the brain have been studied in animals by a number of workers. Macklin and Macklin,[1] Russell [2] and Carmichael [3] showed that the microglia cells, also known as macrophages and gitter cells, are the major participants in the acute reaction which follows mechanical puncture. These phagocytes, which are part of the reticuloendothelial system,[4] slowly clean up the blood and the cellular detritus resulting from the injury. The number and the activity of these cells reach a maximum about the fifth day after injury. It has been shown quantitatively by Hicks and Opie [5] that in the spleen these same phagocytes are capable of active proteolytic digestion of red and white cells, but their ability to digest myelin and other phospholipid-containing materials in the brain has not been demonstrated. It is well known that in the wound tracks of mechanical punctures of the brain phagocytes containing lipids and hemosiderin persist for periods of from weeks and months to almost a year after the initial injury. This phenomenon suggests that these cells are capable of only slow digestion of phospholipids derived from brain tissue and erythrocytes. Many tissues contain enzymes capable of splitting phosphoric acid from its combinations with organic substances.[6] These enzymes have been loosely designated as "phosphatases." Normal brain tissue contains phosphatases which are able to produce slow autolysis. Giri and Datta [7] have demonstrated in the brains of sheep phosphatases which split phosphoric acid from sodium beta glycerophosphate.

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1. Macklin, C. C., and Macklin, M. T.: Arch. Neurol. & Psychiat. **3**:353, 1920.
2. Russell, D.: Am. J. Path. **5**:451, 1929.
3. Carmichael, E. A.: J. Neurol. & Psychopath. **9**:209, 1929.
4. Dunning, H. S., and Furth, J.: Am. J. Path. **11**:895, 1935.
5. Hicks, S. P., and Opie, E. L.: Am. J. Path. **18**:333, 1942.
6. King, E. J.: Biochem. J. **28**:476, 1924.
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It is not feasible to isolate the phagocytes of the brain and measure their phosphatase activity. However, the tissue of a puncture wound rich in phagocytes can be excised and its phosphatase activity compared directly with that of similar normal brain tissue. Any difference between these two may be attributed to the enzyme activity of the phagocytes in the wound track. Another study, in which experimental punctures were made in the brains of 40 mice, which were then killed at varying intervals, showed that the number and the activity of the phagocytes had reached a maximum about five days after the injury, indicating that lesions of this age would present the best conditions for a study of the enzymes of the phagocytes.

Experiments were therefore undertaken in 30 mice to compare the phosphatase activity of wound track tissue five days old with that of normal brain tissue. This activity was studied in regard to both brain tissue substrate and sodium beta glycerophosphate substrate. Since it was necessary to excise some brain tissue along with the wound tracks, this could be measured and made to serve as brain substrate when desired. Sodium beta glycerophosphate, a convenient standard laboratory substrate, contains phosphorus linkages which are analogous to those in phospholipids. Therefore, in some experiments this substance was added to the already present brain substrate in order to magnify the effect of the phosphatase activity. The hydrogen ion concentration of p_H 7.2 at which the experiments were conducted was selected as being within the probable physiologic range of intracellular and extracellular fluids as found in the living brain. The significance of phosphatase activities of brain tissue carried on outside of the physiologic range of p_H 6.5 to 7.5 is doubtful for two reasons: (1) It is not known whether higher or lower concentrations of hydrogen ions occur in the living brain, and (2) certain phosphatases are known to reverse their activities completely with large changes in hydrogen ion concentration, effecting cleavage of glycerophosphate at one concentration and synthesis at another.[8]

EXPERIMENTAL PROCEDURES AND RESULTS

Young adult male and female white mice between the ages of 6 weeks and 3 months were used in all experiments. Under ether anesthesia and aseptic conditions each cerebral hemisphere was punctured from the side with a 20 gage occluded hypodermic needle to a measured depth of 4 mm. The needle was introduced through the scalp and the skull and passed through the meninges and the cerebrum into the interbrain. The animals were killed rapidly with ether five days after injury, and the brains were removed and the enzyme studies started at once. All animals were submitted to autopsy, and none was remarkable except for the puncture wounds.

8. Gortner, R. A.: Outlines of Biochemistry, ed. 2, New York, John Wiley & Sons, Inc., 1938, chap. 37, p. 942.

The sample of brain tissue in which phosphatase activity was to be measured was in each case ground fine in a mortar, suspended in barbital sodium buffer solution (2.12 Gm. of monosodium diethylbarbiturate in 500 cc. of distilled water). When it was desired to add glycerophosphate as a substrate to the already present brain tissue substrate, this was done in the amount of 2.5 Gm. of sodium beta glycerophosphate ($Na_2\ C_3H_5\ [OH]_2\ PO_4 \cdot 5\frac{1}{2}H_2O$) per 500 cc. of barbital sodium buffer.[9] The p_H was adjusted to 7.2 in each case after the suspension was prepared by using 5 per cent hydrochloric acid without effective changes in volume. Bromthymol blue was used as the indicator. Incubation was carried out at 37 C. in stoppered glass vessels, in the presence of toluene to prevent bacterial decomposition. Normal brain tissue for control studies was always taken from an area corresponding to the site of the wound track tissue, namely, the cerebrum. At the beginning of each experiment the initial phosphoric acid present in the suspension of brain tissue was determined as inorganic phosphorus by the colorimetric method of Kolmer and Boerner.[9] This was computed and recorded as milligrams of inorganic phosphorus per gram of brain tissue. In all cases this was found to be from 0.5 to 0.9 mg., a range which is in good agreement with that recorded by Page [10] and Randall.[11] At certain intervals during incubation samples were taken of each suspension and inorganic phosphorus determinations were made. These were also computed and recorded as milligrams of inorganic phosphorus per gram of brain but were corrected by subtracting from them the amount of inorganic phosphorus found in each case at the beginning of the experiments. Accordingly, the recorded values of inorganic phosphorus in the tables represent the actual cumulative increase due to phosphatase activity incubation. Two series of experiments were conducted.

In the first series, the brains of 12 animals—4 normal controls and 8 whose brains had been punctured five days previously—were studied with regard to phosphatase activity toward both brain tissue substrate and glycerophosphate. In each case the entire forebrain was excised and trimmed to weigh exactly 300 mg.; this was then made up to a 10 cc. volume of suspension. This included the wound track if the brain had been punctured. Two of the normal brains and 4 of the punctured brains were thus each made up with plain barbital sodium buffer solution according to the method described. The two other normal brains and the 4 other punctured brains were each made up with barbital sodium buffer to which had been added sodium beta glycerophosphate substrate as described. The amount of inorganic phosphorus liberated by enzyme activity was expressed in milligrams per gram of brain tissue as previously noted. In that group of experiments in which brain tissue was the only substrate the total phosphorus available in 1 Gm. of brain was approximately 3 mg., mostly in the form of phospholipid and therefore available for phosphatase activity.[12] In the group of experiments in which glycerophosphate was added, the calculated volume of suspension which would contain 1 Gm. of brain tissue (33.3 cc.) would have not only approximately

9. The barbital sodium buffer with glycerophosphate is that described for phosphatase and inorganic phosphorus determinations by J. Kolmer and F. Boerner (Approved Laboratory Technic, ed. 2, New York, D. Appleton-Century Company, Inc., 1938, p. 756).

10. Page, I. H.: Chemistry of the Brain, Springfield, Ill., Charles C Thomas, Publisher, 1937, chap. 3, 10 and 11.

11. Randall, L. O.: J. Biol. Chem. **124**:481, 1938.

12. Page.[10] Randall.[11]

3 mg. of phosphorus in the form of phospholipid but also approximately 16 mg. of phosphorus in the form of glycerophosphate. Therefore, if the enzyme action in each group of experiments went to completion, it would level off at about 3 mg. per gram when only brain substrate was available and about 19 mg. when both brain and glycerophosphate acted as substrates. In the experiment the enzyme reactions leveled off when only about half of the substrate was used up in each instance. The results are recorded in tables 1 and 2 and chart 1.

TABLE 1.—*Phosphatase Activity Toward Brain Tissue Substrate by Normal Mouse Brains and Mouse Brains Containing Five Day Old Puncture Wound Tracks*

	Increase in Milligrams of Inorganic Phosphorus Liberated by 1 Gm. of Brain Tissue During 72 Hr. at p_H 7.2 and 37 C.*		
	24 Hr.	48 Hr.	72 Hr.
Normal brain	0.8	1.6	1.5
Normal brain	0.9	1.6	1.6
Brain with 5 day wound tracks	0.6	1.6	1.4
Brain with 5 day wound tracks	0.7	1.5	1.6
Brain with 5 day wound tracks	0.6	1.3	1.2
Brain with 5 day wound tracks	0.6	1.5	1.6

* Total available phosphorus 3 mg. per gram of brain tissue.

If a smooth curve be drawn through the successive values of inorganic phosphorus in each experiment in tables 1 and 2, the difference between normal and punctured brain is not greater than the difference within the normal group or within the punctured group. Therefore there is no significant difference, in these experiments, between the phosphatase activity of normal brain and that of punctured brain.

TABLE 2.—*Phosphatase Activity Toward Brain Tissue Substrate with Added Sodium Beta Glycerophosphate by Normal Mouse Brains and Mouse Brains Containing Five Day Old Puncture Wound Tracks*

	Increase in Milligrams of Inorganic Phosphorus Liberated by 1 Gm. of Brain Tissue During 72 Hr. at p_H 7.2 and 37 C.*			
	6 Hr.	24 Hr.	48 Hr.	72 Hr.
Normal brain	1.7	4.1	7.9	8.1
Normal brain	1.9	4.6	8.4	8.6
Brain with 5 day wound tracks	1.9	4.7	8.7	10.2
Brain with 5 day wound tracks	1.9	5.0	9.5	10.1
Brain with 5 day wound tracks	2.0	4.9	9.1	10.8
Brain with 5 day wound tracks	2.2	5.1	9.2	9.8

* Total available phosphorus 19 mg. per gram of brain tissue.

In order to determine the effect of a change in hydrogen ion concentration within the physiologic range, the concentration in each case shown in tables 1 and 2 was changed from p_H 7.2 to p_H 6.5 at the end of seventy-two hours, and incubation at 37 C. was carried out for an additional twenty-four hours. There was no increases in liberated inorganic phosphorus, indicating that this change of hydrogen ion concentration did not effect a change in phosphatase activity.

Since no significant difference in phosphatase activity between the normal and the injured tissue was revealed in the first series of experiments tabulated in tables 1 and 2 and chart 1, a second series was carried out in which a relatively more concentrated suspension of wound track tissue was used. This was done because the first experiments involved considerable dilution of the tissue of the actual wound tracks. Small differences in the enzyme activity between normal and abnormal tissue might have been obscured by this dilution. In this second series of experiments the brains of 18 animals were used, i. e., 5 normal control brains, 11 brains with wound tracks five days old, and 2 brains which had been punctured just before the animals were killed for the study. These two fresh wound track controls were included in order to rule out the possible phosphatase activity of blood, for even after five days there is still some blood in the wound track. From each brain only 50 mg. of tissue, including the wound track in the cases of puncture

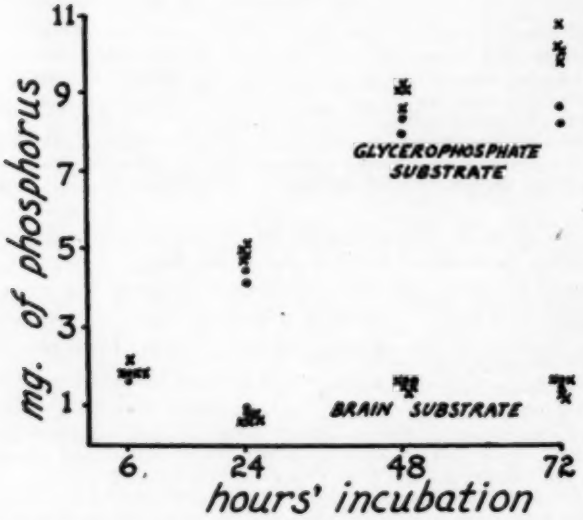

Chart 1.—Graphic presentation of phosphorus determinations on control and experimental mouse brains of the first series of experiments (see tables 1 and 2). Normal brains are represented by ×; punctured brains by •.

of the brain, was excised and made up to only 1 cc. of suspension. The technic was in all other respects identical with that described for the first series of experiments. Three of the normal brain controls, 1 control brain with fresh wound tracks and 6 of the brains with five day old wound tracks were studied for phosphatase activity toward brain substrate only. This brain substrate was provided, as before, by the brain tissue excised. A similar group of the 8 other brains was studied for activities toward brain substrate with sodium beta glycerophosphate added in the manner previously described. The results are recorded in tables 3 and 4. In the group in which brain tissue was the only substrate, the total available phosphorus computed for the volume of suspension containing 1 Gm. of brain was, as before, approximately 3 mg. However, when the glycerophosphate was added, the total available phosphorus in a volume of suspension (20 cc.) which would contain 1 Gm. of brain tissue was

3 mg. from the brain and 10 mg. from the glycerophosphate. Van Slyke [13] has pointed out that in hydrolytic enzyme reactions where there is an excess of substrate as in these experiments the reaction velocity is best observed in the first part of the reaction curve and is proportional to the enzyme activity. Therefore, it was necessary only to measure the inorganic phosphorus liberated in this second series during the first twenty-four hours, for in the first series of experiments the curve of maximum phosphatase activity was shown to be established during the first twenty-four hours. The results are recorded in tables 3 and 4 and chart 2.

TABLE 3.—*Phosphatase Activity Toward Brain Tissue Substrate by Normal Mouse Brains and Mouse Brains Containing Five Day Old Puncture Wound Tracks*

	Milligrams of Inorganic Phosphorus Liberated by 1 Gm. of Brain Tissue During 24 Hr. p_H 7.2 and 37 C.*
Normal brain	0.7
Normal brain	0.5
Normal brain	0.8
Brain with fresh wound tracks	0.8
Brain with 5 day wound tracks	0.8
Brain with 5 day wound tracks	0.9
Brain with 5 day wound tracks	0.9
Brain with 5 day wound tracks	0.9
Brain with 5 day wound tracks	0.9
Brain with 5 day wound tracks	0.8

* Total available phosphorus 3 mg. per gram of brain tissue.

TABLE 4.—*Phosphatase Activity Toward Brain Tissue Substrate with Added Sodium Beta Glycerophosphate by Normal Mouse Brains and Mouse Brains Containing Five Day Old Puncture Wound Tracks*

	Milligrams of Inorganic Phosphorus Liberated by 1 Gm. of Brain Tissue During 24 Hr. p_H 7.2 and 37 C.*
Normal brain	4.2
Normal brain	4.2
Brain with fresh wound tracks	3.6
Brain with 5 day wound tracks	4.6
Brain with 5 day wound tracks	4.4
Brain with 5 day wound tracks	5.0
Brain with 5 day wound tracks	5.4
Brain with 5 day wound tracks	4.8

* Total available phosphorus 13 mg. per gram of brain tissue.

Again as in the first series of experiments there is no significant difference in phosphatase activity between normal brain and brain containing a five day old wound track, despite the relatively high concentration of wound track tissue rich in phagocytes in this series.

COMMENT

The results of the experiments demonstrated that the tissue from a five day old wound track in the mouse brain did not possess a signifi-

13. Van Slyke, D. D., in Nord, F. F., and Werkman, C. H.: Advances in Enzymology, New York, Interscience Publishers, Inc., 1942, vol. 2, p. 33.

cantly greater phosphatase activity than corresponding normal brain. At this time, five days after injury, histologic studies showed that the wound tracks contained a maximum number of phagocytes at peak activity. The inference may be made, then, that the phagocytes do not possess a greater quantity of phosphatases capable of splitting phosphoric acid from myelin, dead brain tissue, erythrocyte phospholipids and sodium beta glycerophosphate than does normal brain tissue. The technical necessity of using the whole wound track tissue, since a method for isolating the phagocytes remains to be accomplished, limits the accuracy of the experiments to the extent that very small differences in enzyme activity cannot be detected. Nevertheless these same cells

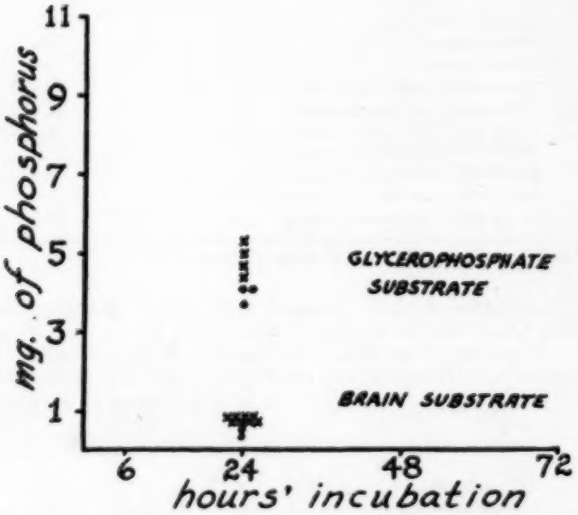

Chart 2.—Graphic presentation of phosphorus determinations on control and experimental mouse brains of the second series of experiments (see tables 3 and 4). Normal brains are represented by ×; punctured brains by •.

when active in the spleen, as noted in a foregoing paragraph,[5] have been shown under similar experimental circumstances to possess marked proteolytic enzymatic activity when phagocytosing red and white blood corpuscles. In such experiments the difference in enzymatic activity between normal spleen and spleen containing large numbers of phagocytes was marked. Thus it would seem that these cells are well equipped to dispose of proteins, but with phospholipids their ability is at best mediocre.

It may be hoped that these experiments will lead to further studies of the enzyme activities of the cells concerned with the response to injury in various tissues. More refined tests for the assay of the

enzymes of the cells of the reticuloendothelial system may be perfected. Such studies are important for the understanding of the fundamental reactions in tissue injury.

SUMMARY

The phosphatase activities of normal mouse brains and mouse brains containing punctured wound tracks five days old and rich in phagocytes, were compared. Both brain tissue (phospholipids) and sodium beta glycerophosphate were employed as substrates.

No significant difference between the phosphatase activity of normal brain and that of wound track tissue was demonstrated.

It was concluded that these brain phagocytes, members of the so-called reticuloendothelial system, are not capable of splitting phospholipids to any noticeable degree. This is found to be in contrast to the proteolytic enzymatic capabilities of this same type of cell.

This sluggish enzymatic activity toward breakdown products of injured brain tissue is in agreement with histologic studies of the brain which show phagocytes containing lipid at sites of injury often persisting for many months.

RENAL INTRATUBULAR SYNCYTIAL MASSES

A Note on Their Origin

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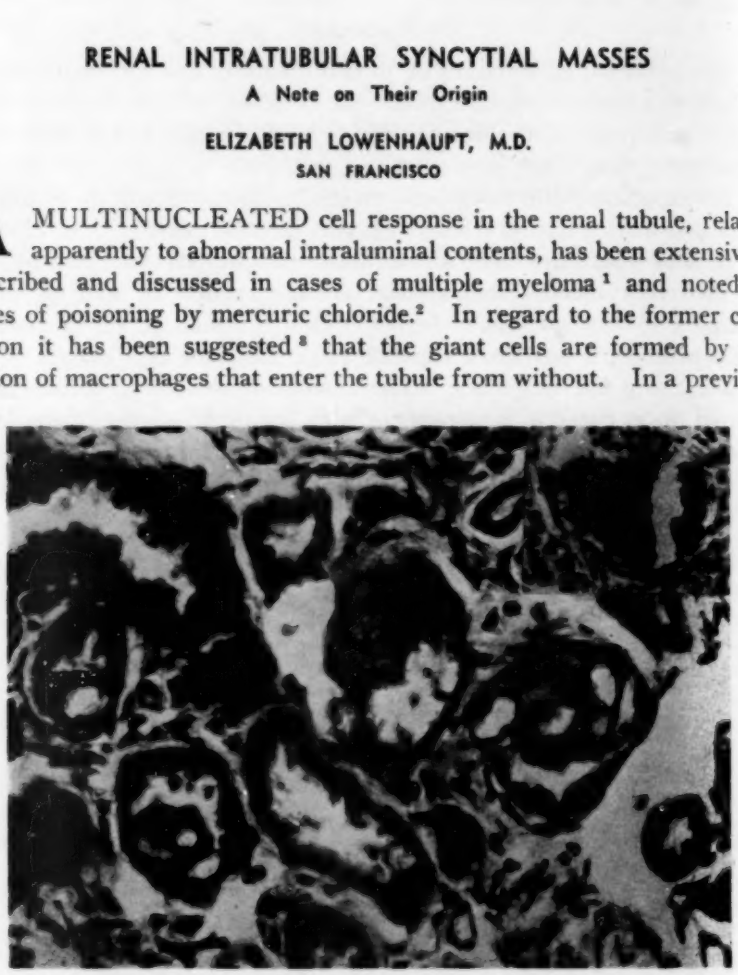
A MULTINUCLEATED cell response in the renal tubule, related apparently to abnormal intraluminal contents, has been extensively described and discussed in cases of multiple myeloma [1] and noted in cases of poisoning by mercuric chloride.[2] In regard to the former condition it has been suggested [3] that the giant cells are formed by the fusion of macrophages that enter the tubule from without. In a previous



Kidney of rat after forty-two days of dietary chloride deficiency. Four tubules are lined by regenerating epithelium, which forms multinucleated cells. In three these cells surround vacuoles, sites of absorbed calcific material (Bouin's fluid fixation). Hematoxylin and eosin; × 420. The inset shows a similar reaction in acetone-fixed tissue, calcific material being present. Gomori's alkaline phosphatase stain; × 115.

From the Department of Pathology, University of California.

1. Forbus, W. D.; Perlzweig, W. A.; Parfentjev, I. A., and Burwell, J. C., Jr.: Bull. Johns Hopkins Hosp. **57**:47, 1935.

2. Harmon, E. L.: Am. J. Path. **4**:321, 1928.

3. Bell, E. T.: Am. J. Path. **9**:393, 1933.

study [4] cells of similar appearance were noted in the kidneys of rats on a chloride-deficient diet and there too appeared related to precipitate within the tubular lumen. Since little comment is found in regard to other possibilities as to the origin of these cell sheets, it seems of interest to illustrate and briefly discuss the lesion.

Material and methods have been described previously,[4] and the figure serves to show the lesion under discussion, which was noted uniformly in the material examined. Four tubules in the illustration are filled with cell masses, which appear to be formed by proliferating cells rather than by desquamation of sheets of degenerating epithelium. This is indicated by the fact that subsidiary tubules are present within each mass and that each of these smaller tubules is lined by flattened cells, suggestive of regenerated tubular epithelium. In addition, cell structures remain well preserved. Likewise, it would appear that these cells are related to foreign material present within the lumen, in contact with them, and perhaps are proliferating in response to this precipitate —indicated by the inclusion of calcific granules within the cell cytoplasm. These points are shown in the figure.

SUMMARY

The observations described suggest that multinucleated structures in renal tubules may arise directly from the tubular epithelial cells, proliferating perhaps in response to contact with abnormal material—in this case calcium.

4. Lowenhaupt, E., and Greenberg, D. M.: Arch. Path. **42**:35, 1946.

MYOCARDIAL GRANULOMAS IN SUBACUTE BACTERIAL ENDOCARDITIS

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IN INSTANCES of subacute bacterial endocarditis, various inflammatory changes have been observed in the myocardium.[1] These changes may be diffuse but are more frequently localized. Commonly, perivascular round cell infiltrations are noted, but true abscesses are rarely encountered. Often, also, small emboli are seen within branches of the coronary arteries, and minute infarcts, either recent or in various stages of organization. If numerous sections are cut from the myocardium, these changes can easily be demonstrated.

In addition to these diffuse or localized inflammatory or vascular changes, granulomas are occasionally encountered. Foremost among them is the Aschoff body. In a previous study,[2] in children, Aschoff bodies were encountered in the myocardium in 14 of 35 hearts with subacute bacterial endocarditis. Libman and Friedberg [3] recently stated that Aschoff bodies are present in about 25 to 45 per cent of cases of this disease. On the other hand, Gelfman [4] reported Aschoff bodies in only 2 of 50 instances of subacute bacterial endocarditis. This discrepancy may be explained by the fact that not all investigators examine a comparable number of blocks from such hearts. These lesions are significant because they constitute the only findings which indicate that the primary lesion of the heart valve was rheumatic in origin.

Aschoff bodies may be present in the various but still recognizable stages. Among 55 instances of subacute bacterial endocarditis, outspoken Aschoff bodies were found in 19. They were more commonly encountered in children and adolescents (under 20 years of age). Among 15 of the latter group they were found in 11. This may be explained by the possibility that relatively more of the myocardium is examined

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This study was aided by a grant from the A. B. Kuppenheimer Fund. This department is supported in part by the Michael Reese Research Foundation.

1. Saphir, O.: Am. J. Path. **11**:143, 1935.
2. Saphir, O., and Wile, S. A.: Am. Heart J. **9**:29, 1933.
3. Libman, E., and Friedberg, C. K.: Subacute Bacterial Endocarditis, New York, Oxford University Press, 1941.
4. Gelfman, R.: Ann. Int. Med. **19**:253, 1943.

histologically in the smaller hearts than in the adult heart if the number of sections examined in both age groups is the same. Aschoff bodies occurring in hearts which are the seat of subacute bacterial endocarditis have been the subject of much discussion. Since a review of such a discussion would lead too far afield, mention may be made only of the recent view of Kelson and White,[5] who stated that two types of relationships appear to be present. Subacute bacterial endocarditis may act as a specific or a nonspecific factor to activate rheumatic fever in susceptible subjects, or subacute bacterial endocarditis may occur during the course of rheumatic fever.

Bracht-Wächter bodies are frequently mentioned as being characteristic of subacute bacterial endocarditis. Perry[6] thought these embolic in nature. In a series of 9 consecutive cases such cellular foci were found in the myocardium in all but 1, and in this case the vegetations were confined to the tricuspid valve. He emphasized that in the earlier stages the cells appear to be polymorphonuclear leukocytes and lymphocytes in almost equal numbers; later the polymorphonuclear leukocytes become fewer and an occasional endothelial cell is seen. Libman and Friedberg[8] stressed that Bracht-Wächter bodies replace the muscles and are not lesions of interstitial tissue. They spoke of localized collections of lymphocytes and mononuclear cells. White[7] stated that Bracht-Wächter bodies had been reported as myocardial lesions more or less typical of subacute bacterial endocarditis. He described them as areas of mononuclear cell infiltration of the interstitial tissue of the myocardium. He further remarked that they are found sometimes in other cardiac infections and that they are not as specific for subacute bacterial endocarditis as are the Aschoff bodies for rheumatic heart infection.

This short review indicates the confusion which prevails in regard to the Bracht-Wächter body. The variation in the descriptions of this structure can easily be explained by the fact that Bracht and Wächter did not describe a single entity. Because of the discrepancies as to just what constitutes a Bracht-Wächter body, it may be of interest to review Bracht and Wächter's original publication in more detail.

Bracht and Wächter[8] studied the hearts of patients who died as a result of acute infectious disease and also those of 4 patients for whom the clinical diagnosis of acute rheumatic arthritis had been made. The purpose of their investigation was to see whether or not Aschoff bodies are found exclusively in rheumatic hearts. They reported the

5. Kelson, S. R., and White, P. D.: Ann. Int. Med. **22**:40, 1945.

6. Perry, C. B.: Bacterial Endocarditis, Bristol, John Wright & Sons, Ltd., 1936.

7. White, P. D.: Heart Disease, ed. 3, New York, The Macmillan Company, 1944.

8. Bracht, E., and Wächter: Deutsches Arch. f. klin. Med. **96**:493, 1909.

finding of Aschoff bodies in 3 of 4 hearts of patients with acute rheumatic fever but in none of the hearts taken from patients who had died of other infectious diseases. Bacteriologic cultures of the blood taken from 2 of the 3 hearts in which Aschoff bodies had been found disclosed diplostreptococci resembling Streptococcus viridans. To see whether or not these streptococci would produce Aschoff bodies, they injected intravenously the streptococci found in each of these cases into 2 rabbits. Into a third rabbit they injected streptococci which were isolated from the heart blood of one of the original 2 rabbits.

Rabbit 1 was given five intravenous injections within forty-eight hours and was killed on the ninth day. Microscopically, minute and small areas of necrosis and cloudy swelling were found in the myocardium. Larger areas of necrosis were surrounded by lymphocytes and fibroblasts, and among the latter were occasional giant cells. Rabbit 2 was given four injections over a period of fourteen days and died spontaneously. There were three minute verrucae at the line of closure of the mitral valve and one on the tricuspid valve. The myocardium disclosed circumscribed areas of cellular infiltrations within the interstitial tissue, occasionally involving the heart muscle fibers. There were also foci of necrosis of muscle fibers and accumulations of lymphocytes, fibroblasts and isolated plasma cells. Rabbit 3 was given four injections over a period of sixteen days and then killed. Verrucae were found on the mitral and the tricuspid valve. Scars were found replacing muscle fibers, and also some calcification was encountered.

From the foregoing review it is clear that only 3 rabbits were used. In rabbits, however, myocardial changes of the type described may occur spontaneously.

For control experiments Bracht and Wächter used 3 rabbits. Two of the rabbits were given intravenous injections of streptococci obtained from a paronychia and 1 rabbit was given intravenous injections of streptococci isolated from infected tonsils. Microscopically, circumscribed areas with central necrosis infiltrated by polymorphonuclear leukocytes were found in the myocardium of these rabbits (abscesses).

From a critical review of the study published by Bracht and Wächter it is clear that the authors worked with no material obtained in cases of subacute bacterial endocarditis. They worked with only a few animals of a species in which myocardial changes occur spontaneously. Their "series" of experiments was carried out on 3 test rabbits and 3 control rabbits. Their findings were definitely not uniform. In view of this it is difficult to comprehend why in the literature the term "Bracht-Wächter bodies" was ever applied to myocardial lesions in subacute bacterial endocarditis. This is particularly confusing since these authors nowhere mentioned myocardial changes in subacute bacterial endocarditis.

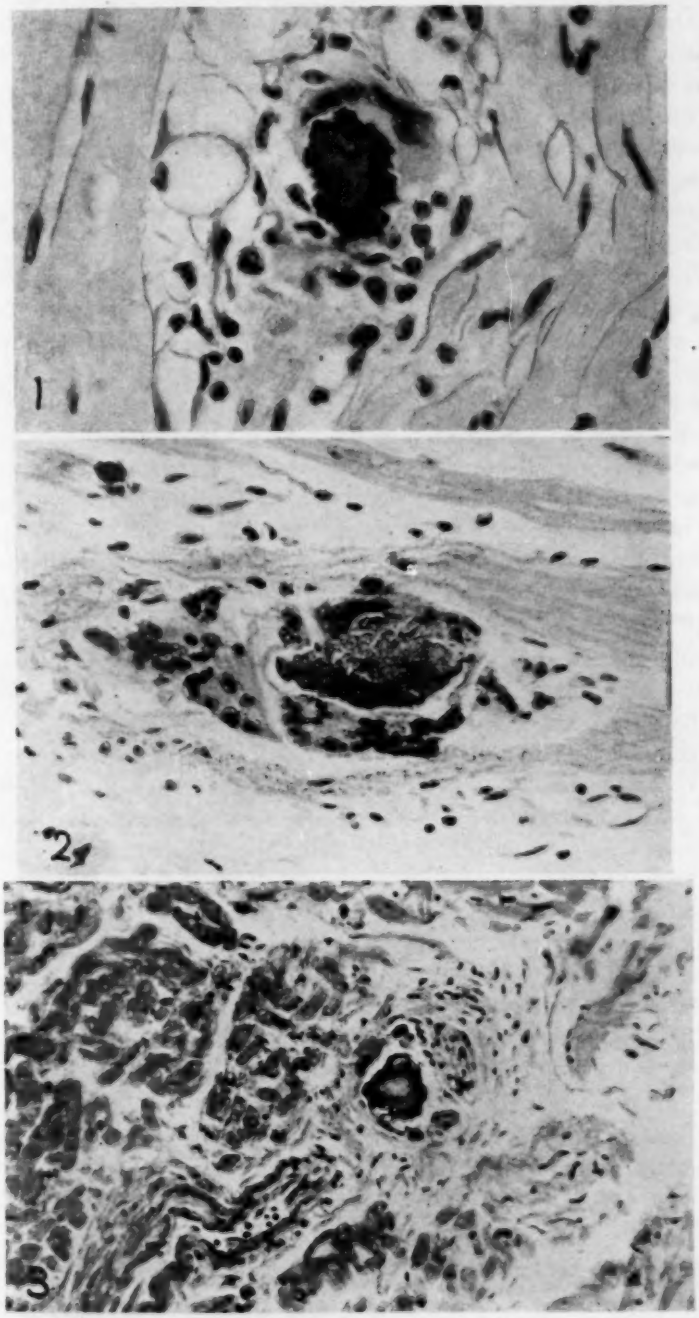

Fig. 1.—Dark amorphous material surrounded by foreign body giant cells. Hematoxylin-eosin preparation; × 580.

Fig. 2.—Calcific material surrounded by giant cells situated in the interstitial tissue of the myocardium. Van Gieson preparation; × 380.

Fig. 3.—Foreign body granuloma with the calcium deposit in the center. Hematoxylin-eosin preparation; × 150.

The majority of communications which discuss Bracht-Wächter bodies and which have appeared since the original study was published show structures which correspond to those illustrated by figure 6 of the original report. These are designated as Bracht-Wächter bodies. Interestingly enough, figure 6 depicts what the original authors named "experimentally produced myocarditis in rabbits following injections of streptococci taken from paronychia." It demonstrates principally areas infiltrated by polymorphonuclear leukocytes.

It is thus clear that Bracht-Wächter bodies in the sense of granulomas specific for, or even characteristic of, subacute bacterial endocarditis do not exist, and that what have been described as Bracht-Wächter bodies are a variety of nonspecific lesions which perhaps may be produced experimentally by intravenous injections of streptococci. What Bracht and Wächter have described has nothing to do with subacute bacterial endocarditis. In a previous communication it has been stressed that the term "Bracht-Wächter bodies" should be discarded.[1] DeVasquez[9] also has favored dropping the term. A study of 15 additional cases of subacute bacterial endocarditis further discredits the use of the term "Bracht-Wächter bodies."

In recently observed instances of subacute bacterial endocarditis, granulomas of a different variety have been encountered in the myocardium. Within the centers of these a small amount of a dark blue–stained (hematoxylin and eosin preparation) amorphous material was found, which stained black with silver (von Kossa). This material, obviously containing calcium, was surrounded by giant cells of the foreign body type and endothelial leukocytes. Only few lymphocytes were seen at the farther periphery. These granulomas, though principally present in the myocardium, were also found in the epicardium adjacent to the myocardium. Occasionally they were surrounded only by endothelial leukocytes and a few lymphocytes. In seemingly early instances this bluish amorphous material could be seen within capillaries or arterioles. A search for bacteria within this amorphous material gave negative results. From this description it is clear that these granulomas were foreign body granulomas, each surrounding a calcific particle.

It is of interest that these granulomas have not been disclosed by previous studies of the myocardium in cases of subacute bacterial endocarditis. They were found in 4 recent instances. The 4 patients had died during the last three years and had been treated extensively with various sulfonamide compounds and with penicillin. Subacute bacterial endocarditis of the mitral and aortic valves was found in all patients at autopsy. Evidence of healing in the form of organizing and organized vegetations of the aortic valve and the formation of

9. DeVasquez, S.: J. Path. & Bact. **49**:33, 1939.

peculiar healing and healed erosive mycotic aneurysms of the mitral valve (to be the subject of a future study) were encountered. It may be merely mentioned that examination of the vegetations of the aortic

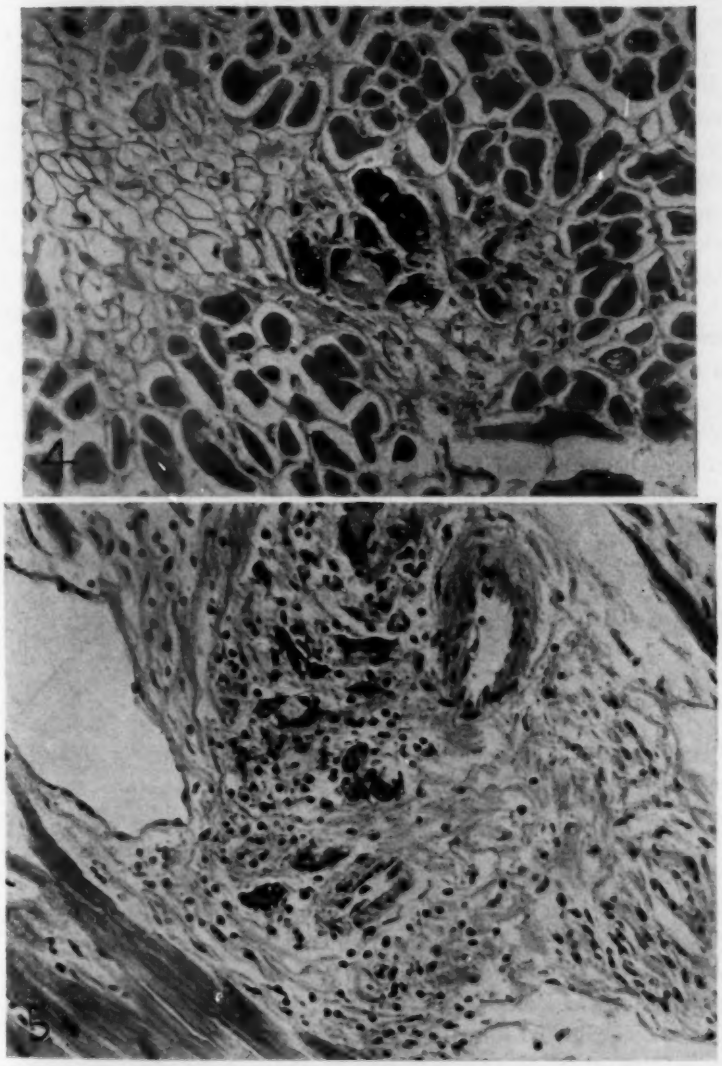

Fig. 4.—Dark amorphous material, some of which is situated in the interstitial tissue. Hematoxylin-eosin preparation; × 250.

Fig. 5.—Calcific material, a few foreign body giant cells and lymphocytic infiltrations situated in the interstitial tissue. Iron-hematoxylin-eosin preparations; × 300.

valve disclosed the presence of granulation tissue at their base and the absence of bacteria. At the periphery of the vegetations patches of

necrosis with few bacteria were noted in addition to smaller or larger amounts of a bluish amorphous material which histochemically was calcium. Only an insignificant amount of granulation tissue was observed in such areas. Thus the outstanding findings were: organization at the base of the vegetations, and necrosis and calcific changes at the free margin.

Because of the fact that precipitated calcium is present within those portions of the vegetations which still show necrosis and little granulation tissue, particles can break loose easily and cause the formation of emboli. Morphologically it seems that necrosis, few bacteria and the simultaneous precipitation of lime salts are characteristic of instances in which therapeutic agents have altered the pathologic appearance of subacute bacterial endocarditis.

Emboli are encountered frequently in branches of the coronary arteries in subacute bacterial endocarditis. As a matter of fact, it had been noted previously that minute infarcts of the myocardium in various stages of healing were the most frequent and perhaps the most commonly encountered single change. The emboli so far described were clumps of bacteria and fibrin or particles from vegetations. None of these emboli calls for the formation of a foreign body granuloma. However, in those instances in which the embolus consists of lime salts, a foreign body reaction in the form of a foreign body granuloma ensues. It might be mentioned that in one of these 4 instances a similar foreign body granuloma was encountered in the kidney.

SUMMARY

A study of the myocardium in instances of subacute bacterial endocarditis disclosed, in addition to diffuse inflammatory changes, two types of granulomatous lesions. One of these, the Aschoff nodule, was found in 19 of 55 instances of subacute bacterial endocarditis. It was present in the myocardium of 11 of the 15 children studied. The other is a foreign body granuloma, obviously caused by calcific deposits, the result of calcific emboli arising from healing vegetations of the aortic valve. These were found only in recently observed instances of subacute bacterial endocarditis, in patients who had been treated with sulfonamide compounds or penicillin.

A critical consideration of the literature on Bracht-Wächter bodies indicates that they signify no definite entity and that, therefore, the term "Bracht-Wächter body" should be discarded.

ORGANIZED EMBOLI OF THE TERTIARY PULMONARY ARTERIES

An Unusual Cause of Cor Pulmonale

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AND
EDWARD F. BLAND, M.D.
BOSTON

CHRONIC COR PULMONALE is the result of long-standing strain of the right ventricle secondary to circulatory obstruction in the lung. The usual causes are emphysema, fibrosis and silicosis, but occasionally primary alterations of the intima or the media of the pulmonary arteries are responsible. A considerable number of cases falling in the latter etiologic group have been studied in this hospital during the past twenty years, and their clinical course is to be reported in the near future. However, one of these is unique from a pathologic standpoint and warrants a separate report.

D. C., a housewife aged 44, was under observation in the Massachusetts General hospital and in the outpatient clinic at frequent intervals during the nine years of her slowly progressive and ultimately fatal illness. In childhood she had measles, mumps, whooping cough and chickenpox. At the age of 29, appendectomy and salpingo-oophorectomy on the right side incident to a tubal pregnancy were done at another hospital. There were no postoperative complications. At the ages of 31, 33 and 35, respectively, she had normal pregnancies and delivered her offsprings at home, without showing cardiovascular symptoms.

Shortly after the last pregnancy, cyanosis of the lips was noted. This sign persisted with slowly increasing intensity throughout the remaining eight years of the patient's life.

At the age of 36 she began to have intermittent sharp pain in the epigastrium and lower substernal region, lasting from a few minutes to several hours, usually associated with a dry cough, and after a few months dyspnea on effort became evident and she entered the hospital. The significant findings were: orthopnea, slight cyanosis of the lips, no clubbing, normal blood pressure, no cardiac murmurs but an abnormally loud pulmonary second sound, right axis deviation and large P waves by electrocardiogram (fig. 1), and enlargement of the right ventricle and of the pulmonary conus by roentgenogram, without enlargement of the auricles.

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Circulatory studies supplied the following data:

		Normal
Circulation time		
Saccharin, arm to tongue	51.6 seconds	20
Ether, arm to lungs	20.6 seconds	10
Crude pulmonary	11.0 seconds	
Venous pressure (arm)	125 mm. of saline solution	
Red cell count	6,000,000	
Hemoglobin	113 per cent	
Arterial blood (determinations by John H. Talbott)		
Alveolar carbon dioxide–combining power	22.0 mm. of mercury	40
Arterial carbon dioxide–combining power	22.4 mm. of mercury	40
Oxygen capacity	26.1 volume per cent	20
Saturation	93.4 per cent	95
Cell volume	57.9 per cent	42
Total carbon dioxide	43.4 volume per cent	58
p_H	7.54	7.40

It is of interest that the circulation rates suggested obstruction proximal to the lungs, whereas the blood gas studies slightly favored congenital heart disease.

The subsequent seven years were characterized by slowly increasing failure of the right side of the heart, manifested by progressive cardiac enlargement, per-

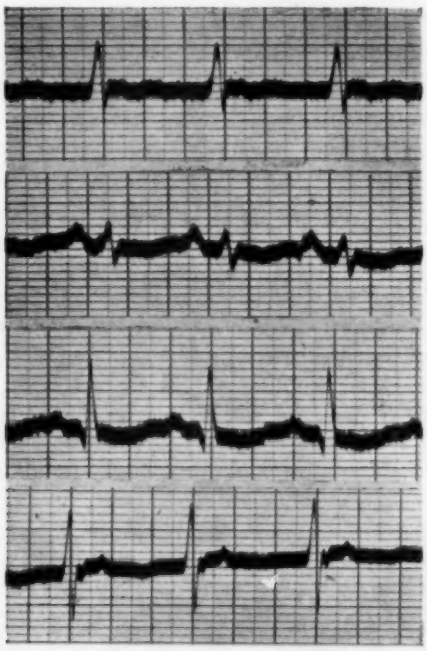

Fig. 1.—Electrocardiogram (leads I, II, III and IV) showing right axis deviation and low T waves.

sistent gallop rhythm, venous distention, swelling of the liver and peripheral edema, requiring digitalis and diuretic therapy and occasional hospitalizations for more intensive treatment.

During the final year of the patient's life she required mercurophylline injection, U. S. P., twice a week to control the failure of the right side of the heart. The lungs remained clear throughout the illness (fig. 2). A painful umbilical hernia developed and added considerably to the patient's discomfort. Ultimately, operation with the region under local anesthesia was performed, but the wound

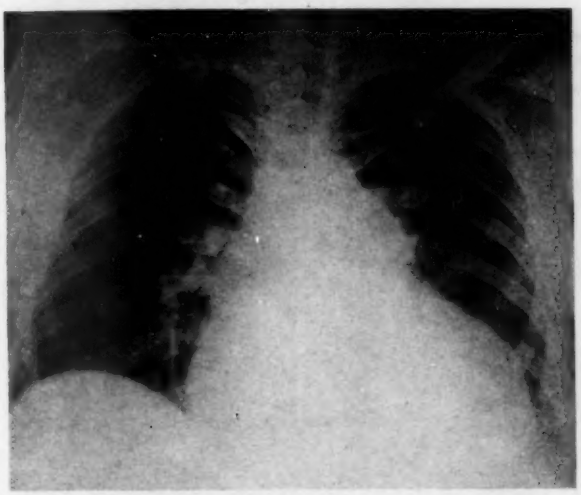

Fig. 2.—Roentgenogram made twelve days before death, showing marked enlargement of the heart. The heart measures 18.3 cm.; the thorax, 30.0 cm.

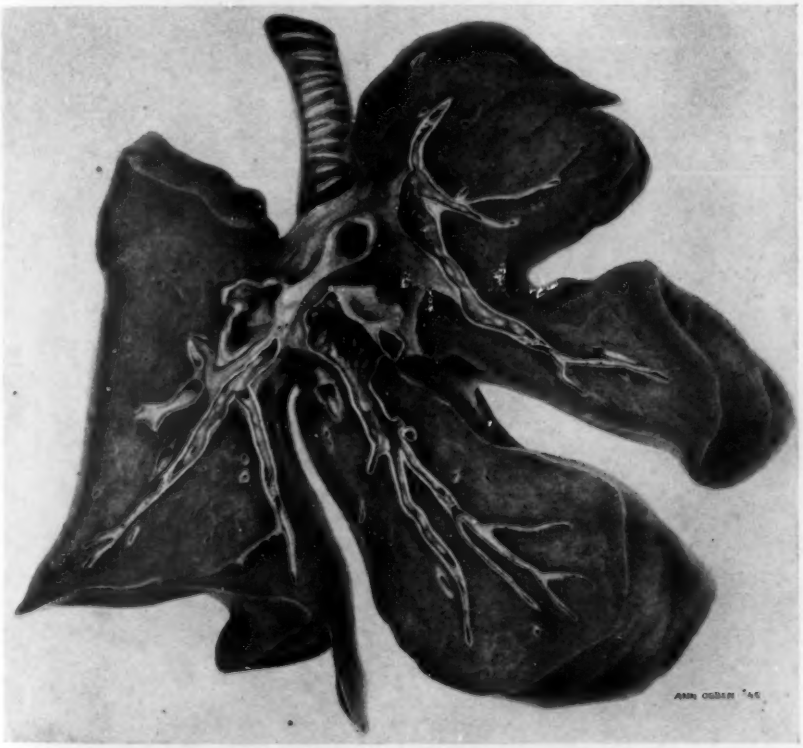

Fig. 3.—Drawing of a section of the right lung showing lacelike occlusio of tertiary branches of the pulmonary arteries. Note the thickness of the wall proximal to the obstruction.

disrupted on the third day, and she weakened progressively and died on the thirteenth day, nine years after the onset of her initial cyanosis. The terminal illness was complicated by ileus, mild jaundice and persistent failure of the right side of the heart.

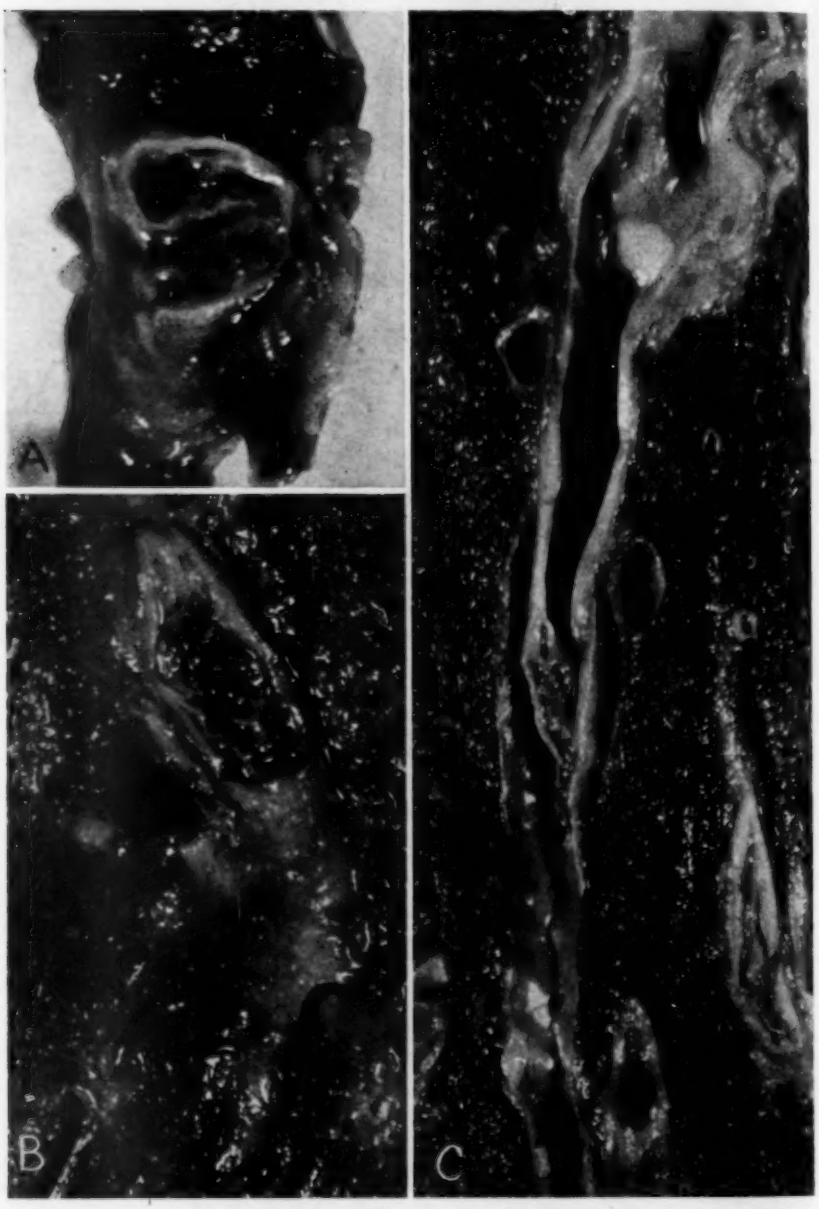

Fig. 4.—*A* and *B*, cross sections through points of obstruction, showing the latticework appearance of the fibrous trabeculae. *C*, photograph of one of the pulmonary arteries shown in figure 3. The atheromatous thickening proximal to the obstruction is conspicuous in contrast to the thin wall distal to the occlusion.

The postmortem examination showed that the immediate cause of death was acute general peritonitis.

The heart was enlarged, weighing 520 Gm. There were marked dilatation and hypertrophy of the right auricle and ventricle, the latter measuring from 6 to 10 mm. in thickness. The left ventricle was small, and its wall measured only 10 to 12 mm. The valves were normal. The coronary arteries and the aorta showed only slight atheromatous changes.

The unusual findings were in the pulmonary arteries. Practically every tertiary branch was occluded between 2 and 4 cm. from its origin by a meshwork of thin tough fibrous trabeculae 5 to 10 mm. in length (fig. 3). On cross section they had a latticework appearance, but water could not pass the obstruction even under considerable pressure (fig. 4 *A* and *B*). In the interstices of some of these occluding lesions was clotted blood, most of which was clotted post mortem, but some

Fig. 5.—One of main pulmonary arteries showing a valvelike pocket of the intima (arrow). Note also the cross section of one of the occluded tertiary branches (double arrows).

was clotted definitely ante mortem and organized. The main pulmonary artery, its primary and secondary branches, and those portions of the tertiary branches proximal to the obstructions were thick, and the intima was covered with atheromatous plaques. In sharp contrast, the vessel walls beyond the lesion were thin, atrophic and completely free from atheroma (fig. 4 *C*). This difference was so striking that the exact point of obstruction in each vessel could be predicted on sectioning the lung; as long as the vessel wall was thick and atheromatous, the obstruction was still distal to the section level. In one of the main pulmonary arteries there was a small valvelike semilunar pocket of the intima, measuring 7 to 8 mm. in diameter, with its opening away from the pulmonary valve (fig. 5). This lesion was reminiscent of the so-called accessory aortic cusp occasionally seen below the aortic valve in long-standing aortic regurgitation.

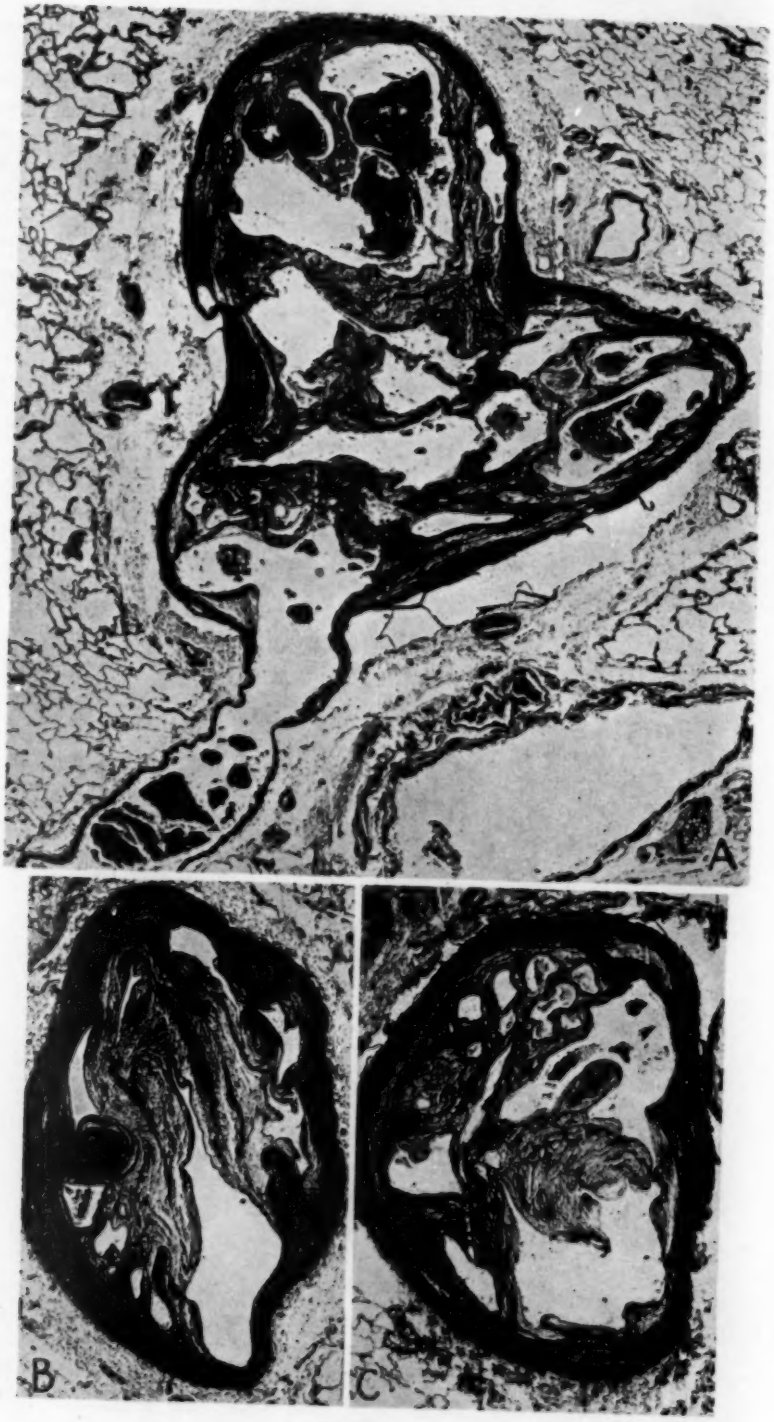

Figure 6

(See legend on opposite page)

Microscopic examination of these meshworks showed that they were composed of fibrous tissue in which occurred numerous vascular spaces, varying in size and lined with endothelium. Some of these channels were filled with fresh blood; others, with organized thrombi; still others were empty. Elastic tissue preparations revealed that not only was the wall of the main vessel composed of dense elastic fibers, but a large amount of elastica was present throughout the walls of the small vascular channels within the lumen. Many of these apparently intraluminal vessels showed extensive fibrous intimal proliferation. The entire picture of these lesions is consistent with organized and recanalized thrombi.

The vessels proximal to the obstructions showed extensive fibrous intimal proliferation, but their lumens were not diminished to any appreciable extent. The obstruction in the tertiary branches was not a gradual narrowing but a strikingly abrupt process. Beyond the obstruction the vessels that grossly appeared thin and atrophic in contrast to the proximal thick portion were actually thicker than normal when viewed microscopically, and a few showed slight fibrous intimal proliferation. Some of the smaller arteries in and just beneath the pleura showed marked medial hypertrophy.

Another interesting finding was the presence of several pleural fibrous plaques, 1 to 3 cm. in diameter, which extended into the parenchyma for distances of 3 to 10 mm. Some of these were puckered and dimpled on the surface. Grossly and microscopically they presented the characteristic appearance of the healed infarcts previously described by one of us (B. C).[1]

The liver weighed 1,800 Gm. and showed well advanced cardiac cirrhosis.

COMMENT

When this patient was first seen in the Massachusetts General Hospital eight years before she died, she already had well marked clinical and laboratory evidence of cor pulmonale. Thus we can assume that the initiating pathologic cause probably occurred at least some years before that time.

One interpretation of the findings in the pulmonary arteries is to consider them as advanced arteriosclerosis of a primary nature. In fact, Rosenthal,[2] in a report of 3 cases of sclerosis of the pulmonary artery, in one of which (case 3) the arterial changes were not unlike those in our case microscopically, did believe that his case was merely an instance of severe arteriosclerosis. If this were true of our case, it would be difficult to explain why this overgrowth of nodular vascular tissue should occur only in the tertiary branches at points relatively equidistant from the hilus.

1. Castleman, B.: Arch. Path. **30**:130, 1940.
2. Rosenthal, S. R.: Arch. Path. **10**:717, 1930.

Explanation of Figure 6

Photomicrographs (elastic tissue preparations) of cross sections through obstructions, showing the extensive organization and recanalization. Note the absence of sclerosis or of extension of the thrombus into a branch in *A*.

The almost constant location of the obstructing lesions suggested that perhaps they were the result of some embryologic maldevelopment; such as a rete mirable, but Dr. J. Lewis Bremer, emeritus professor of anatomy at the Harvard Medical School, studied the material and was unable to account for these lesions on a developmental basis.

This leaves two further possibilities—organized and recanalized thrombi or emboli. If thrombi, they would almost certainly have been formed on the basis of arteriosclerosis, but why always in the tertiary branches? One would have to assume that the patient had idiopathic sclerosis of the pulmonary arteries long before the development of the obstructing lesions. This is a definite possibility, namely, that a long-standing sclerosis slowly narrowed the lumen to such an extent that thrombi would be prone to develop and more so in the smaller tertiary branches.

A more plausible explanation is that the lesions were the result of the lodging of small emboli in the tertiary branches, which then were organized and recanalized. The recanalization, however, was not sufficient to allow much, if any, blood to flow through most of the lesions. Since the same-sized small pulmonary arteries were involved, one would have to assume that the emboli came from thrombi in small vessels, the most likely source being the pelvic veins or possibly those of the lower part of the legs. Unfortunately, these veins were not investigated at autopsy by the prosector. It seems more likely that emboli would lodge in approximately the same-sized vessel than that thrombi would develop on the basis of the previous arteriosclerosis always in a tertiary branch and never in a primary or a secondary one. It is unlikely that these emboli were produced by amniotic fluid with epithelial squamae as has been reported by Steiner and Lushbaugh,[3] because in those cases the capillaries and very small arteries were occluded and not the main tertiary branches.

We should like to suggest, therefore, that some years before the patient first entered the hospital, perhaps after the pelvic operation or one of the pregnancies, thrombi developed in the pelvic veins, which began to shower the lungs with emboli. Some of these emboli went far enough out in the lung to produce infarcts, since healed infarcts were found at autopsy, but most of them were sufficiently proximal to the pleura to allow collateral circulation and thus prevent infarction. The pulmonary pressure proximal to the emboli was thus increased and led to the severe atherosclerosis in contrast to the relative absence of atheroma beyond the obstruction. The dilatation and slight atheroma of the vessels beyond the obstruction can be accounted for by the increased pressure in the collateral circulation, part of which must have come

3. Steiner, P. E., and Lushbaugh, C. C.: J. A. M. A. **117**:1245 and 1340, 1941.

from the bronchial arteries. Evidence for the latter's role in the collateral circulation is the marked thickening of the pleural and subpleural arteries which are derived from the bronchial arteries.

SUMMARY

A 44 year old woman presented clinical evidence of cor pulmonale and slowly progressive heart failure for nine years. Postmortem examination showed localized occlusion of almost every tertiary pulmonary artery by what were believed to be organized and recanalized emboli. Evidence of marked hypertension in the pulmonary circuit was the severe atherosclerosis that ended abruptly in each vessel at the point of obstruction.

ABSENCE OF THE LEFT CARDIAC VENTRICLE WITH APLASIA OF THE AORTIC ORIFICE AND HYPOPLASIA OF THE AORTA

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BROOKLYN

TOTAL absence of the left ventricle in man is so rare a condition that no instance is included in the list of 1,000 cases of cardiac anomalies in Abbott's Atlas.[1] The only comparable instance is one mentioned by Krumbhaar.[2] This unreported case was observed at the University of Pennsylvania Hospital by Dr. W. F. Sheldon (no. 41-1249). As in my case, the heart had no trace of the left ventricle or of the mitral or the aortic valve. Unlike the heart in my case, it had a large foramen ovale. Walls [3] reported a case in which one ventricle gave rise to a well developed aorta as well as to a normal pulmonary artery. In this, as in other instances of biatrial, trilocular hearts, there is a common or undivided ventricle rather than a solitary right or a solitary left ventricle. While simple arrest of development is an adequate explanation for the persistence of a common or undivided ventricle, there is no satisfactory explanation for true absence of the left ventricle. "Detorsion defects" such as were discussed by Shapiro [4] do not seem adequate.[5] Intrauterine endocarditis is a possible explanation, particularly in the presence of demonstrable inflammatory changes.[6] In my case, however, there was no trace of inflammation or of scarring.

REPORT OF A CASE

A full term white girl was born at the Bayonne Hospital by low forceps extraction on June 26, 1944, at 4:35 a. m. Because she was cyanotic, she was given a breathing mixture of oxygen and carbon dioxide. Respirations remained labored, and at 8:10 a. m., three hours and thirty-five minutes after birth, she died. The mother, 28 years old, had had a normal child previously. The progress of her labor in the present case had been entirely normal. The fetal heart rate before

From the Department of Pathology, Bayonne Hospital and Dispensary, Bayonne, N. J.

1. Abbott, M. E.: Atlas of Congenital Cardiac Disease, New York, American Heart Association, 1936.
2. Krumbhaar, E. B.: J. Mt. Sinai Hosp. **8**:737, 1942.
3. Walls, E. W.: Lancet **2**:668, 1941.
4. Shapiro, P. F.: Arch. Path. **9**:54, 1930.
5. Bremer, J. L.: Arch. Path. **34**:1016, 1942.
6. von Zalka, E.: Frankfurt. Ztschr. f. Path. **30**:144, 1924.

birth had been 130 per minute. Owing to the short period of postnatal observation, no clinical diagnosis was made.

At necropsy, eight hours after death, the child weighed 8 pounds, 3 ounces (3,714 Gm.) and was well developed and well nourished. The skin was pale. There were a few drops of clear fluid in the pericardial cavity. The pericardial surfaces were smooth and shiny. The heart measured 4.5 cm. from base to apex and 4 cm. across the base and weighed approximately 22 Gm. The apex was blunt and was made up of the right ventricle. The right atrium was about 2 cm. wide; the endocardium was smooth and shiny. The superior and inferior venae cavae and the coronary sinus entered in the usual way. There was no trace of a foramen ovale, nor was there any other atrial septal defect. The wall of the right atrium varied between 0.1 and 0.2 cm. in thickness; the wall of the auricle was thinner. The right atrioventricular orifice was 5 cm. in circumference; the three leaflets were delicate.

The right ventricle formed the bulk of the heart. Its wall varied between 0.3 and 0.7 cm. in thickness, including the trabeculae carneae. The papillary muscles were slightly thickened. The pulmonic orifice was 2.8 cm. in circumference, and the three leaflets were delicate. Two of them appeared to be anterior and one posterior. The orifices of the right and left pulmonary arteries were in the postero-lateral aspects of the main vessel and were located, respectively, 0.2 cm. and 0.7 cm. above the pulmonic orifice. The intimal surface of the pulmonary artery was smooth and shiny. Where the widely patent ductus arteriosus opened into the aorta, its intimal surface was wrinkled and finely pitted and its wall was slightly thickened. The pulmonary artery continued as the ductus arteriosus into the descending aorta, the level of transition being marked by the arch of the hypoplastic aorta opening into the descending aorta. The entire segment from the level of this communication down to the level of the left branch of the pulmonary artery is to be considered the ductus arteriosus. This is shown in figure 9 of Popják's [7] report of a case of atresia of the aorta and hypoplasia of the left ventricle.

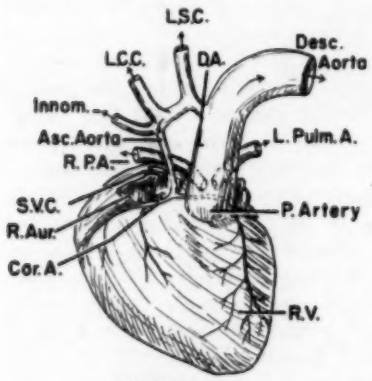
The left atrium was 0.3 cm. wide and 0.7 cm. long. The endocardium was finely trabeculated, and the wall was 0.3 cm. thick. The left auricle was small and well formed. Entering the left atrium were two right and three left pulmonary veins. The upper right vein entered the atrium in the usual way. The lower right vein entered through a common orifice shared by the uppermost of the three left veins. The middle left vein entered the atrium independently in the same manner as the upper right. The lowermost left vein, having the same caliber as the others up to the wall of the atrium, entered by means of a pinpoint orifice. There was no trace of a mitral valve or of the left ventricle. The aorta began, without a trace of aortic valve leaflets or any vestige of endocardial cushions, at the level of the origin of the coronary arteries. The right coronary artery ran between the right auricle and the right ventricle and descended along the right border of the latter. The left coronary artery curved around the base of the pulmonary artery and divided into two branches at the level of the left auricle, supplying, respectively, the left border and the posterior aspect of the right ventricle. The ascending portion of the aorta was 0.2 cm. wide externally, and its intimal surface was smooth. Its origin was almost directly behind the base of the pulmonary artery. From there it ran, with a slight obliquity, to the right and upward. One and one-half centimeters from its origin, it gave rise to the innominate artery, which was 0.4 cm. wide, then to the left common carotid artery, 0.3 cm. wide and then,

7. Popják, G.: J. Path. & Bact. **54**:67, 1942.

0.6 cm. beyond, to the left subclavian artery, also 0.3 cm. wide. Just beyond this it joined the ductus arteriosus and the descending aorta. Only beyond the ductus did the aorta broaden considerably, reaching a diameter of 0.7 cm. (1.7 cm. internal circumference), approximately the size of the pulmonary artery. The intercostal arteries arose in the usual manner from the thoracic aorta.

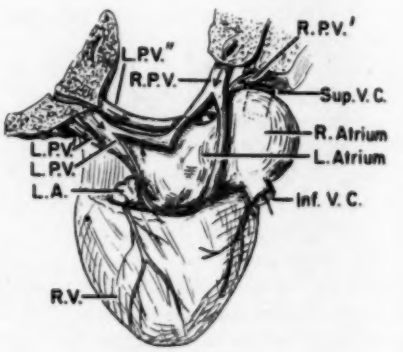
The lungs were buoyant, and each had the usual number of lobes. The thymus weighed 12 Gm. and the spleen 15 Gm. The stomach, the duodenum and the small and large intestines were not unusual. The liver was dark brown and weighed 150 Gm. The pancreas, the adrenal glands and the kidneys were of the usual size. The pelvic organs were not remarkable. The brain weighed 350 Gm. and presented nothing of note.

Microscopically, there was no evidence of inflammatory reaction in the heart. The area of junction between the right ventricle and the two atriums was occupied by hyalinizing fibrous connective tissue. There was no scarring in the adjacent myocardium or elsewhere.



ANTERIOR

Anterior view of the heart: *R.V.*, right ventricle; *Cor. A.*, right coronary artery; *R.Aur.*, right auricle at margin of right atrium; *S.V.C.*, superior vena cava; *R.P.A.*, right branch of pulmonary artery; *Innom.*, innominate artery; *L.C.C.*, left common carotid artery; *L.S.C.*, left subclavian artery; *D.A.*, ductus arteriosus; *Desc. Aorta*, descending aorta; *L. Pulm. A.*, left branch of pulmonary artery; *P. Artery*, pulmonary artery.



POSTERIOR

Posterior view of the heart: The left atrium is open. There is neither mitral valve nor left ventricle. *R.V.*, right ventricle; *L.A.*, left auricle; *L.P.V.*, left middle pulmonary vein; *L.P.V.'*, left lower pulmonary vein with pinpoint orifice in atrium; *L.P.V."*, left upper pulmonary vein, crossing to the right, to enter the atrium in a common orifice shared with the lower right pulmonary vein; *R.P.V.*, upper right pulmonary vein; *R.P.V.'*, lower right pulmonary vein; *Sup.V.C.*, outline of superior vena cava on anterior aspect of right atrium.

In a preparation of tissue taken through the wrinkled portion of the ductus arteriosus, the intima was uneven, broad and loose. The thickening was due to an increase of both elastic and collagenous fibers. The media was uneven and, in places, narrow. The elastic laminas were scanty and uneven and were often interrupted by an increase of collagenous connective tissue. The adventitia was broader than the media and narrower than the intima. It was composed of heavy laminas of elastic fibers interspersed with collagenous fibers. Preparations of the aorta were not remarkable.

In the lungs the interalveolar capillaries were markedly distended with blood. The connective tissue of the bronchial walls was slightly increased and sparsely infiltrated with small round cells, large mononuclear cells and polymorphonuclear leukocytes. The bronchial capillaries were markedly distended with blood. The lumens of the bronchial veins were considerably widened and their walls thickened. The pulmonary veins, especially with the Van Gieson stain, were the most conspicuous feature of the preparations of the lungs. They were extraordinarily enlarged, and their walls were greatly thickened. The bulk of the thickening was composed of many laminas of elastic fibers between the slightly thickened intima and the slender adventitia. Compared with the veins, the pulmonary arteries were inconspicuous. In the arteries, the intima was slender, the media thickened slightly and the adventitia thickened and hyalinizing. In most instances the adventitia equaled or exceeded the media in thickness. Only vascular congestion was noted in the other organs.

Anatomic Diagnosis.—Anomaly of the heart: absence of the left ventricle and of the mitral and aortic orifices; hypoplasia of the aorta; patent ductus arteriosus; hypertrophy of the right ventricle and of pulmonary and bronchial veins; passive congestion of viscera.

COMMENT

Any comment as to the mode of origin of the curious defects described is apt to arouse controversy without giving material aid; hence, none will be ventured. Concerning the circulation of the blood, this may be said: The dead end in the left atrium was apparently compatible with life for a few hours. Consequently there must have been some return of blood from the left side of the heart to the right. In view of the enlargement of the bronchial and pulmonary veins it seems safe to assume that there was a communication between the two systems. Even normally "a considerable quantity of the blood which is carried to the lungs through the bronchial arteries is returned to the left side of the heart through the pulmonary veins."[8] In addition, there was a tidal flowing of blood back and forth through the lungs, with all the enlarged pulmonary veins being used as a pulsating expansion chamber. In each systole of the ventricle this venous expansion chamber was distended by aerated blood. In each diastole the elastic retraction of the heavy walls of these veins forced the aerated blood back into the pulmonary and bronchial capillaries, where it was mixed with the nonaerated blood coming through the pulmonary arteries. Of course, the blood in the systemic circulation was even less aerated than was this mixture, for it consisted largely of blood that had passed directly from the right atrium and ventricle into the aorta and great vessels without having entered the lungs at all. Considering the feeble aeration of this system, one finds it remarkable that the infant survived as long as she did.

8. Gray, H.: The Anatomy of the Human Body, ed. 24, edited by W. H. Lewis, Philadelphia, Lea & Febiger, 1943, p. 669.

IN VITRO EFFECT OF PENICILLIN ON ENDAMOEBA HISTOLYTICA

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STUDIES on Endamoeba histolytica [1] have been an important activity of the Department of Parasitology and Bacteriology of the Michael Reese Hospital since the epidemic of amebic dysentery that occurred in Chicago in 1934. When a member of the department began to culture Penicillium notatum and harvest the crude penicillin, it was but natural that its effect should be tried on E. histolytica as well as on various bacteria. Observations were made on wet mounts of E. histolytica to which penicillin was applied directly and on cultures of the parasite treated with serial dilutions of the crude penicillin. No inhibitory action on wet preparations or on cultures of E. histolytica was observed. The study was temporarily discontinued because it did not appear feasible to use crude penicillin, and the purified product was unavailable.

In the latter part of 1944 a symposium on amebiasis was given at the Royal Society of Tropical Medicine and Hygiene.[2] The participants unanimously agreed that there was urgent need for investigations of new chemotherapeutic agents in the treatment of amebiasis, because none of the drugs in current use was without toxic properties, and none assured cure. Hargreaves,[3] Manson-Bahr [4] and Willmore [5] had independently used penicillin in treatment in cases of refractory amebiasis and had noted that clinical improvement of the patients was spectacular but that their stools still contained E. histolytica, and that when peni-

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This study was aided by a grant from the Committee on Scientific Research of the American Medical Association.

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2. Adams, A. R. D.: Tr. Roy. Soc. Trop. Med. & Hyg. **38**:237, 1945; Lancet **2**:752, 1944.

3. Hargreaves, W. H.: Tr. Roy. Soc. Trop. Med. & Hyg. **38**:244, 1944.

4. Manson-Bahr, P.: Tr. Roy. Soc. Trop. Med. & Hyg. **38**:251, 1944.

5. Willmore, J. G.: Tr. Roy. Soc. Trop. Med. & Hyg. **38**:257, 1944.

cillin therapy was discontinued, the clinical symptoms recurred. In a later publication Hargreaves [6] reported the use of penicillin and succinyl sulfathiazole followed by a specific drug as the most effective treatment in refractory cases of amebiasis. In this report he stated, without submitting data, that neither sulfathiazole nor penicillin was effective for amebas in the laboratory.

We recalled our early attempts to inhibit amebas by the addition of crude penicillin in vitro and decided to repeat and expand our initial experiments, using purified penicillin.

DIRECT MICROSCOPIC PROCEDURE

Two similar wet preparations of feces teeming with motile E. histolytica were prepared for microscopic examination. A drop of penicillin was added to one mount and a drop of saline solution to the other so that the concentrations of the two were similar. Different portions of the mounts were examined to ascertain that approximately equal numbers of motile amebas were present in each mount. These preparations were observed on the warm stage at five minute intervals for two or three hours. The amebas remained motile, and no change in their activity was noticed. On a number of occasions one typical ameba of the penicillin-treated wet mount was kept in the field and watched for an hour. At the end of the hour this ameba always retained its motility; pseudopods were still being extruded, and morphologically it appeared normal. This direct application of solutions containing from 5 to 10,000 Oxford units of penicillin per cubic centimeter to specimens of stools containing motile amebas and of twenty-four hour positive cultures was repeated a number of times, with negative results.

CULTURAL PROCEDURE

The effect of penicillin on cultures of E. histolytica was tested by making serial dilutions of a penicillin standard (100, 500, 1,000 and 5,000 Oxford units per cubic centimeter) and adding known quantities per cubic centimeter to the liquid portion of Cleveland's [7] medium (1 part of serum to 6 parts of isotonic solution of sodium chloride, p_H 7 to 7.2). The penicillin levels of the finished medium corresponded roughly to blood levels obtained in patients treated for various bacterial infections; i. e., the levels varied from a trace, 0.03 unit, to 20 units or more. Each tube, containing 4.5 cc. of liquid culture medium, was inoculated with 0.5 cc. of a uniform suspension of emulsified stool or with 0.5 cc. of a uniform suspension of stool culture. The cultures were incubated at 37 C. and examined at intervals of twenty-four, forty-eight and seventy-two hours for motile amebas, for level of penicillin, for proportion of gram-positive to gram-negative organisms and for hydrogen ion concentration (p_H). A number of strains of E. histolytica, including one from the Army, and diverse types of stool specimens were cultured, namely, fecal material in which cysts of E. histolytica predominated, fecal material containing trophozoites and cultures and subcultures teeming with motile amebas. For comparison, similar tests were run, in which sulfadiazine, carbarsone, or chiniofon was substituted for penicillin. Sulfadiazine was used on the supposition that it would suppress gram-negative flora, in contrast to the suppression of gram-positive flora by penicillin, and thereby indicate the effect of different

6. Hargreaves, W. H.: Lancet **2**:68, 1945.
7. Cleveland, L. R., and Collier, J.: Am. J. Hyg. **12**:606, 1930.

types of bacterial flora on the viability of amebas. Carbarsone was included as a control because it was the drug most commonly used by the staff of the Michael Reese Hospital in the treatment of amebiasis. Chiniofon was used because it acts directly on amebas as demonstrated by its general use in retention enemas. Control cultures of E. histolytica were always included to prove the viability of the strain used. In some series of cultures the penicillin level decreased until there was only a trace at the end of twenty-four or forty-eight hours. Because of this, a series of cultures was set up in which the level was maintained at a constant by adding penicillin at intervals and checking the level. Some of the drugs used are more effective in an alkaline medium, and one set of cultures was alkalinized, p_H 7.5, and studied for divergent results.

Effect of Penicillin on Cultures of Four Strains of Endamoeba Histolytica

	Tube 1*	Tube 2	Tube 3	Tube 4	Tube 5	Tube 6	Control Tube
Strain 1	0.4 unit	1 unit	2 units	3 units	4 units	6 units	0
24 hr. reading	++	++	++	++	++	++	++
48 hr. reading	++	++	++	++	0	0	++
Strain 2 (Army strain)	2 units	5 units	10 units	15 units	20 units	30 units	0
24 hr. reading	++	++	++	++	++	0	++
48 hr. reading	++	++	++	++	0	0	++
Gram-positive flora	75%	50%	40%	35%	25%	50%	50%
p_H	6.5	7.0	7.0	6.5	6.5	6.5	6.5
Strain 3	4 units	10 units	20 units	30 units	40 units	60 units	0
24 hr. reading	++	++	++	++	++	++	++
48 hr. reading	++	++	++	++	++	0	++
Gram-positive flora	50%	60%	50%	50%	50%	10%	50%
p_H	7.0	6.5	7.0	6.5	6.5	6.5	6.5
Strain 4 (cysts)	4 units	10 units	20 units	30 units	40 units	60 units	0
24 hr. reading	++	++	++	++	++	++	++
48 hr. reading	++	++	++	++	++	++	++
Gram-positive flora	10%	10%	10%	30%	40%	40%	60%
p_H	7.0	6.5	7.0	6.5	6.5	6.5	6.5

* Each tube contained 4.5 cc. of diluted serum and 0.5 cc. of amebic inoculum. The given amount of penicillin, in Oxford units per cubic centimeter, was added to the medium before addition of the inoculum.

++ = motile amebas; + = a few motile amebas; ± = precysts; 0 = negative for amebas.

The table illustrates the results of subjecting four strains of E. histolytica to various concentrations of penicillin. Only an occasional inhibitory effect was observed, regardless of whether the inoculum was feces, culture or subculture, or whether it contained cysts or trophozoites, as long as it was rich in amebas. Similar sets of cultures treated with sulfadiazine did not show any effect on the viability of the amebas. Chiniofon and carbarsone tended to inhibit the growth of E. histolytica. Alkalinizing the cultures had no apparent effect on the amount of growth. The set of tests in which the penicillin level was maintained at a constant showed no appreciable effect on the viability of the amebas. The proportion of gram-positive to gram-negative flora in the amebic cultures had little uniformity, regardless of the addition of either penicillin or drugs. However, in examining smears, it was noted that there were always a few of those that had been subjected to penicillin in which there were a suppression of

gram-positive organisms and a predominance of gram-negative organisms even up to 70 to 95 per cent. The sulfadiazine-treated cultures reversed these findings. Carbarsone and chiniofon were irregular in their effect on the fecal flora. In all cultures the p_H varied from 6 to 7.5, a range in which E. histolytica is viable.

RESULTS AND CONCLUSIONS

Wet mounts of emulsions of feces teeming with E. histolytica subjected to penicillin varying in concentrations from 5 to 10,000 units per cubic centimeter still contained motile amebas at the end of three hours.

Cultures of E. histolytica, either cysts or trophozoites, were in most instances unaffected by penicillin even when the penicillin levels were maintained as high as 30 Oxford units per cubic centimeter for forty-eight hours.

Penicillin tended to suppress gram-positive bacteria in cultures.

The fecal flora, either normal or abnormal, had no apparent effect on E. histolytica.

Variation in the hydrogen ion concentration of cultures from p_H 6 to p_H 7.5 did not influence the viability of E. histolytica.

In vitro studies indicated that penicillin had little or no effect on either the trophozoites or the cysts of E. histolytica. By inference it seems probable that the improvement observed in the clinical symptoms of patients with amebiasis under penicillin therapy must be due to the action of the drug on the secondary bacterial invaders of the tissues.

SOME EFFECTS OF PROLONGED MASSIVE ESTROGEN TREATMENT ON THE RAT

With Special Reference to the Thymus

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THE CYSTIC epithelial structures developing in the thymus after prolonged massive treatment with estrogenic substances were reported in 1941 by Ross and Korenchevsky.[1] Their report was confirmed by me in 1944.[2] An extension of these observations, described in the present account, affords additional information on the development and the structure of these abnormal growths.

The typical histologic pattern of the thymus of a young and healthy animal, with its sharp demarcation into cortex and medulla, is readily modified by a wide variety of conditions. The so-called age involution or, in the younger animal, the accidental involution resulting either from pathologic conditions or from a variety of stimuli (Selye[3]) causes pronounced and characteristic atrophic changes in the appearance of the thymus. The distinction between cortex and medulla is lost, lymphocytes disappear, and the parenchyma is invaded and replaced by connective tissue.

It has been demonstrated repeatedly that injections of estrogens will produce rapid involution of the thymus. The nonspecificity of the many agents or factors producing thymic atrophy and the uniform appearance of the involuted thymus, irrespective of its cause, are well known. It was thus of special interest to discover that conspicuous epithelial cysts could be produced in an atrophic thymus simply by increasing the dose of the estrogen and extending the treatment.

EXPERIMENTAL PROCEDURE

Twenty-three adult albino rats (Sprague-Dawley) were used. Twelve were treated continuously for periods varying from one month to ten and one-half months with estradiol dipropionate, which was injected weekly in doses of either 0.1 or 0.2 mg. (0.1 mg. in 0.1 cc. of sesame oil). The remaining 11 animals either were left untreated or were treated with a volume of sesame oil equivalent

From the Department of Anatomy, University of Illinois College of Medicine.

The estradiol dipropionate used in this investigation was supplied by Ciba Pharmaceutical Products, Inc., through Dr. Ernst Oppenheimer.

1. Ross, M. A., and Korenchevsky, V.: J. Path. & Bact. **52**:349, 1941.
2. Plagge, J. C.: Anat. Rec. **89**:537, 1944.
3. Selye, H.: Brit. J. Exper. Path. **17**:234, 1936.

to that administered to the experimental rats. As far as possible the direct comparisons of test and control animals were made between litter mates. The ages at the beginning of the injection periods ranged from 2 to 6 months. All of the animals except 2 were females; 2 of the test females and 1 of the controls were spayed at the time treatment was begun. However, neither sex nor castration modified the effects of excessive estrogen treatment.

During the course of the treatment the animals were weighed once a week, and any changes in general health or in color and texture of hair were noted. At autopsy the thymus was removed quickly, weighed in a milligram torsion balance and fixed immediately in Zenker's solution to which solution of formaldehyde U.S.P. had been added in the concentration of 5 per cent. Tissues were embedded in paraffin, sectioned at 8 microns and stained with hematoxylin-eosin-azure II.

RESULTS

As in the experiments described in previous reports, in which three to four months was the extent of treatment with massive doses of estrogens, conspicuous large and small epithelium-lined cysts appeared in the thymus (fig. 1). Usually the cells lining the lumens were irregular in size and shape, but occasionally they were columnar or cuboidal and fairly well organized, resembling either a simple or a stratified epithelium (figs. 2 and 3).

A striking characteristic of the cyst was the material contained within its lumen. There was an apparent secretion that stained in a variety of colors, from pale pink, through various shades of blue, to a deep purple. It was common for two colors of secretion to be present within the same cyst. When this occurred and the cysts were numerous, their follicular appearance was suggestive of the thyroid gland.

Actual evidence that this material was being secreted into the follicle was never obtained in fixed preparations. However, lining the cysts were numerous cells which at their distal ends had cytoplasm that took the same red and blue colors as the cyst cavity. There were also occasional small solid nests or cords of cells with colored cytoplasm that were not associated with an actual cyst. This observation suggests the beginning of a new cyst. In addition, there were occasional isolated single epithelial cells containing the same colored material.

In some cysts filled with a pink secretion numerous blue droplets were present. It is possible that the blue material had been secreted more recently.

When the cysts were small, they tended to be isolated; when they were large, they were likely to be interconnected by cords or tubes. They always ended blindly and were surrounded either by the parenchyma or the connective tissue of the thymus.

Debris, consisting primarily of degenerating cells, was frequently present within the secretion of the cysts. This intrafollicular material appeared to be of epithelial origin, although degenerating lymphocytes

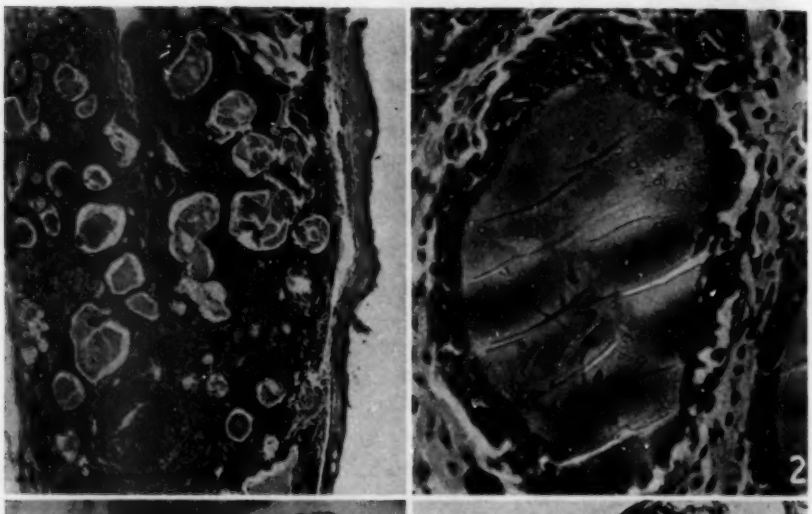

Fig. 1 (rat treated by weekly injection of 0.2 mg. of estradiol dipropionate for ten and a third months).—Epithelium-lined cysts pervade the entire area of the section of thymus. One remaining island of lymphocytes appears near the bottom of the figure. × 57.

Fig. 2 (rat treated by weekly injection of 0.2 mg. of estradiol dipropionate for nine months).—Small thymic cyst showing a tendency toward irregular stratification of epithelium. × 382.5.

Fig. 3 (rat treated by weekly injection of 0.1 mg. of estradiol dipropionate for four months).—Epithelium of a thymic cyst. Note the distention within the cytoplasm at the lower left side. × 875.5.

Fig. 4 (rat treated by weekly injection of 0.2 mg. of estradiol dipropionate for ten and a third months).—Development of a large thymic cyst. Portions of epithelium are being pinched off into the cavity. Fluid and cellular debris can be seen within the cysts in this figure and also in the others. × 57.

of the thymus were occasionally observed. In certain favorable sections desquamation of the outer layer of the stratified epithelium was seen. In other instances, in which cyst formation was extensive, small islands of epithelium appeared to have been pinched off and set free within the cavity of the cyst (fig. 4).

The relative size and the number of cysts were approximately proportional to the length of treatment. The thymuses of animals that had been treated with estrogens for ten months were depleted almost completely of lymphocytes, and the epithelial neoplasms were much more numerous than in those with only four months' injection. However, the total size of the thymus was further reduced by approximately 50 per cent in the rats that had been treated for the longer period. Thus it was not possible to produce a true tumor in the thymus by extending the period of treatment to approximately two and a half times the duration reported previously. The pathologic condition was exaggerated, but the thymus was reduced in size.

It has been reported by Marine [4] that epithelial structures resembling those described here are found occasionally in the thymuses of most animals, most commonly in that of the dog. Ross and Korenchevsky [1] have implied that such structures were rare in their control rats. Only in 3 of 81 normal controls did the thymus contain a "few small undeveloped nests of epithelial cells." Similarly, Selye,[3] who observed the induction of tubular epithelial structures in the thymus of the rat forty-eight hours after subcutaneous treatment with morphine, has never seen such formations in the thymus of a normal rat. In the present series, in which 11 animals served as either untreated or sesame oil–treated controls, the thymuses of 6 animals were studied in serial sections. Small epithelial cysts could be found by careful searching in all the control thymuses examined. A few of these cysts approached the development of the smallest cysts found in the experimental animals (figs. 5 and 6). The injection of pure sesame oil had no influence on the occurrence of the structures.

In an attempt to determine the origin of the cysts a small series of animals was subjected to colchicine treatment following preliminary massive estrogen stimulation of one, two or three months' duration. A colchicine dose of 0.1 mg. per hundred grams of body weight of rat was injected eight hours before the rat was killed and examined. There was no apparent accumulation of mitotic figures following the terminal application of colchicine.

The first indication of cystic development was usually seen in the medulla of the thymus (fig. 7). In the estrogen-treated animals solid cords of epithelial cells pervaded this area, becoming increasingly

4. Marine, D., in Cowdry, E. V.: Special Cytology, New York, Paul B. Hoeber, 1928, vol. 1, sect. 17, p. 549.

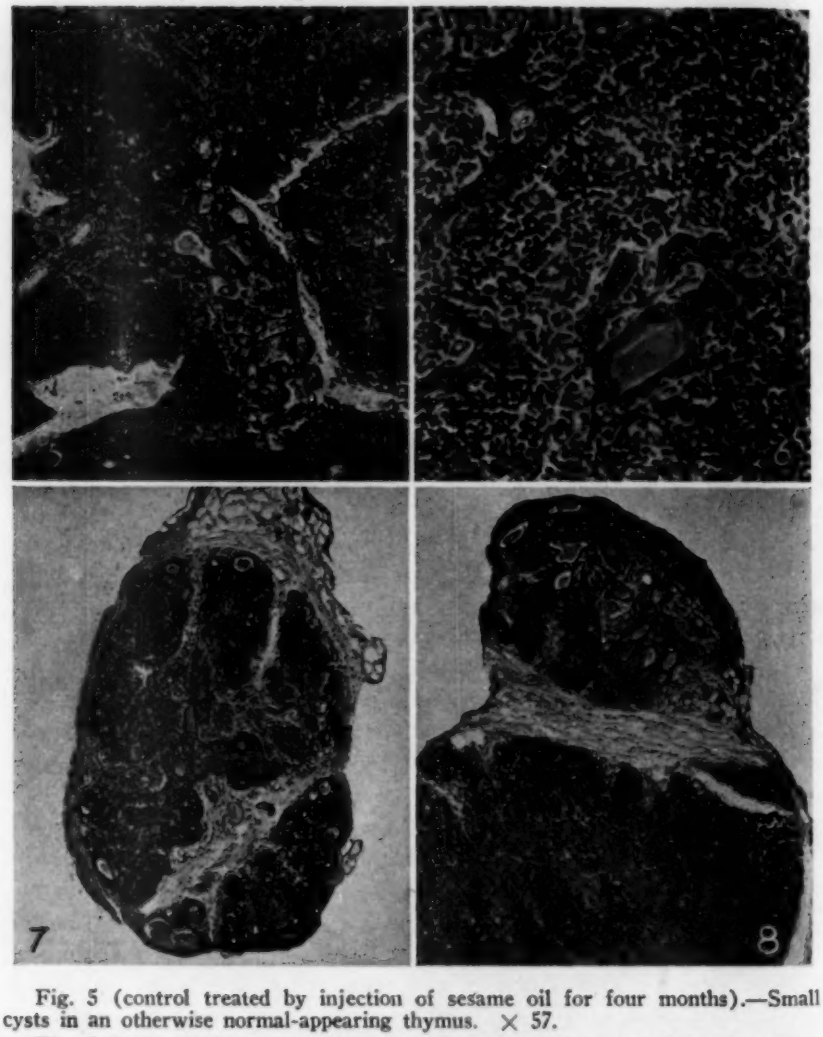

Fig. 5 (control treated by injection of sesame oil for four months).—Small cysts in an otherwise normal-appearing thymus. × 57.

Fig. 6 (control given no treatment).—The epithelial cysts within the parenchyma of the thymus are similar to the larger cysts of the estrogen-treated animals. × 229.5.

Fig. 7 (rat treated by weekly injections of 0.2 mg of estradiol dipropionate for three months).—The early development of epithelial cysts is primarily in the medullary portion of the thymic lobule. × 57.

Fig. 8 (rat treated by weekly injection of 0.2 mg. of estradiol dipropionate for three months).—Section through both lobes of the thymus; the smaller area, with many cysts, is from the cranial end of one lobe; the larger area, from the other lobe, represents a more caudal region and contains only a few cysts and epithelial cords. × 57.

prevalent as an anastomosing network, within the meshes of which lymphocytes were trapped. Concomitant with the loss of distinction between cortex and medulla, the cords invaded the area of the cortex. Simultaneously a few small cysts frequently appeared in the connective tissue, entirely isolated from the parenchyma of the thymus. In the later stages the cords developed lumens, many of which ballooned into large spheric cysts.

If a large and growing cyst encountered any resistant structure, such as a large blood vessel, it usually forked and wrapped itself around the structure.

Serial sections of the thymus showed that the cysts did not occur uniformly throughout the organ. Marine [4] observed that the cranial extremities of the lobes were the favored location for their origin. In order to test this conclusion in the present experiment care was exercised to distinguish the cranial and the caudal ends of the thymus during the preparation of the sections.

Cysts occurred throughout the thymus but were found more often at the ends of the organ. Usually the cranial end had more cysts than the caudal end. Figure 8 shows a cross section through both lobes of the thymus. The two lobes were oriented in such a way that the section includes an area from the cranial end of one lobe and from a more central region of the other lobe. The smaller area at the top of the picture, showing many cysts, is from the cranial end of one lobe. The larger area, from the other lobe, represents a more caudal region and contains only a few cysts and epithelial cords. It is well infiltrated with lymphocytes, and the distinction between cortex and medulla is typically sharp.

In the most severely altered thymuses only traces of lymphatic areas remained. These were usually located peripherally in the lobes, but now and then a resistant nodular island of normal-appearing thymus tissue remained (fig. 1).

Although this report is intended to deal principally with thymic effects, other extragenital alterations were observed as a result of the long-continued massive injections of the estrogen. These will be treated briefly in this report.

The more prolonged periods of injection were frequently terminated prematurely by the occurrence of pyometra and occasionally by infections of the respiratory tract and other complications. Injections continued for three or four months stimulated the uterus without causing an infection, but severe pyometra developed when the injections were continued for several more months. The condition apparently came on abruptly, judged from the sudden and rapid decrease in body weight and the general weakened condition of the animal. Although the injections were to have been continued for at least one year, ten and a

half months was the longest period of treatment possible under these conditions. Because of the high frequency of this infection and because of its possible secondary influence on the involution of the thymus, a few animals were killed while still healthy. The induction of pyometra following estrogenic treatment has been observed by numerous investigators (Kaufmann and Steinkamm [5]; Gardner [6]; Hale and Weichert [7]; Nelson [8]).

The body weights of the estrogen-treated animals, taken routinely at weekly intervals, were appreciably less than those of their litter mate controls. The weight of the estrogen-treated animal usually remained at a plateau during treatment whereas that of the control gradually increased. This suppression of growth confirms earlier reports by Forbes,[9] Hooker and Pfeiffer [10] and Bogart, Lasley and Mayer.[11]

During the course of treatment the fur of the rats was observed for any alterations. Forbes,[12] several months after subcutaneous implantation of pellets of a crystalline estrogen, noticed partial pigmentation of the fur and partial alopecia. In the present experiment no color change of hair attributable to estradiol dipropionate was noted although the lengths of treatment and the amounts of the estrogen given corresponded in general to those employed by Forbes.[12] However, a brownish tipping of the hair, particularly on the scruff of the neck, which appeared quite similar to that described by Forbes,[12] developed in a few rats suffering from respiratory tract or other infection whether or not they were given injections. It is not assumed that this coloring is in any way related to the overdose of estrogen, since healthy rats had a snowy white coat after eight or ten months of continuous treatment. The discrepancy in results, since the dosage was comparable, may be due to differences in strains of rats or to a more constant rate of absorption with implantation of pellets. Interference with hair growth, however, was definitely observed. In shaved areas the hair grew out at about one-half normal rate in animals receiving injections. This substantiates an observation made in rats by Hooker and Pfeiffer [10] and a recent report by Williams, Gardner and DeVita [13] of experiments in which estrone was applied locally on dogs.

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5. Kaufmann, C., and Steinkamm, E.: Arch. f. Gynäk. **162**:553, 1936.
6. Gardner, W. U.: Cancer Probl., Symposium, Science, 1937, supp., vol. 85, p. 67.
7. Hale, H. B., and Weichert, C. K.: Proc. Soc. Exper. Biol. & Med. **55**: 201, 1944.
8. Nelson, W. O.: Yale J. Biol. & Med. **17**:217, 1944.
9. Forbes, T. R.: Endocrinology **30**:761, 1942.
10. Hooker, C. W., and Pfeiffer, C. A.: Endocrinology **32**:69, 1943.
11. Bogart, R.; Lasley, J. F., and Mayer, D. T.: Endocrinology **35**:173, 1944.
12. Forbes, T. R.: Endocrinology **30**:465, 1942.
13. Williams, W. L.; Gardner, W. U., and DeVita, J.: Endocrinology **38**:368, 1946.

Daily vaginal smears demonstrated that estrogen treatment produced a three to four week period of diestrus, followed by continuous estrus. Occasionally at the beginning of treatment a few days of estrus occurred before the diestrus period. The prolonged diestrus period following excessive administration of estrogen in normal female rats was first observed by Selye, Collip and Thomson[14] and is associated with large transitory corpora lutea in the ovary.

During the process of obtaining vaginal smears 4 of the estrogen-treated females assumed a momentary lordosis, similar to the mating response. One female responded this way on seventeen of thirty days.

COMMENT

In this experiment an attempt was made to produce a true tumor or cancer in the thymus of a rat by giving massive doses of estrogen for a period of ten and a half months. At the end of this time there was no indication of a carcinoma. On the contrary, the thymus was further reduced in size by involution. But the atypical cystic growths, observed after treatment of three to four months, were considerably more extensive. Inflammatory developments in the uterus, terminating in severe pyometra and other complications, precluded further extension of treatment.

While the experiments reported here confirm the previously published contention that excessive estrogen treatment is responsible for the development of an extensive growth of epithelial cords, tubes and fluid-filled sacs in the thymus, a thorough histologic analysis of the thymuses of untreated litter mate controls revealed in a majority of cases similar, though much smaller, epithelial structures.

It appears that estrogens influence the thymus in two ways. In the first place, they cause typical involution—decrease in size and loss of lymphocytes. Continued treatment produces the extensive epithelial cysts. Estrogens are thus capable of producing an atrophic condition in the thymus and subsequently inducing active growth of an epithelial nature.

Although it has not been possible to determine the origin of the epithelial network, it is probable that the reticulum is involved, especially since the structures are usually first detected in the medulla of the parenchyma and rarely in the connective tissue. The epithelial nature of these pathologic structures recalls the embryologic origin of the thymus from the pharyngeal pouches. Hassall's corpuscles are so rare and so poorly developed in the thymus of the rat that it is unlikely that they are associated with abnormal growths. However, Steiner,[15]

14. Selye, H.; Collip, J. B., and Thomson, D. L.: Proc. Soc. Exper. Biol. & Med. **32**:1377, 1935,

15. Steiner, P. E.: Proc. Soc. Exper. Biol. & Med. **49**:62, 1942.

who induced similar epithelial hyperplasia in the thymus of the guinea pig by direct implantation of pellets of methylcholanthrene, suggests that at least some of the cysts produced in the thymuses of his guinea pigs were actually enlarged Hassall's bodies.

SUMMARY

Extensive epithelial cords and cysts, containing fluid, have been produced in the thymus of the rat after prolonged massive treatment with estradiol dipropionate. Tumors failed to develop even after ten and a half months of continuous injections. Further treatment had to be abandoned because of the development of pyometra, infections of the respiratory tract and other complications leading to death. Cystic structures were most pronounced at the ends of the thymus, particularly at the cranial extremity.

Small epithelial structures, similar to the larger ones of the test animals, were found in the majority of the controls,

Body weight, hair growth and the estrus cycle were also modified by the prolonged and excessive treatment with estrogen.

MEDIAL CALCIFICATION OF ARTERIES OF INFANTS

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SCHENECTADY, N. Y.

THE GENESIS of arteriosclerosis has occupied the minds of scientific workers for more than a century. Among the various theories on this subject, none has found wider acceptance or remained in general favor over a longer period than the senescence theory. In recent years more and more investigators have refused to accept arteriosclerosis as a natural involutionary process of aging and have looked on it as a disease initiated by some form of injury of the arterial wall followed by sequence of changes.

Arteriosclerosis occurring in infants, although rare, is of considerable interest. While the arteriosclerosis of the adult very likely is in most instances a complex product brought about by action of several agents to which the vessels were subjected in the course of life, it is obvious that less complicated causal and developmental conditions prevail in infants showing arteriosclerosis.

Following is a report of extensive medial calcification of arteries in an infant 10 weeks old. Data on similar lesions in infants are summarized in the accompanying table.

REPORT OF A CASE

A 10 week old baby girl was admitted to the hospital on September 8 because of general debility and wasting.

The infant was born at full term to healthy white parents. The father was 20 and the mother 17 years of age. The Wassermann reaction of the blood of each parent was negative. The mother had been well during pregnancy and had not received vitamins or any form of medication. The labor and the delivery were uneventful. At birth the infant measured 47 cm. in length and weighed 2.5 Kg. No cyanosis, convulsions or abnormalities were noted. The hospital record showed that the infant took the feedings well. The weight, after the usual decline in the first few days, reached the initial birth weight on the seventh day. The mother and the baby left the hospital after eight days. The history of the child's development from the time of discharge until the time of reentry is incomplete. On questioning, the mother stated that the infant presented no difficulties in the first five weeks. It was fed on a formula of evaporated milk and water (1:3). The bowel movements were normal, and the baby cried little. In the sixth week a physician was consulted, who suggested that 5 drops of percomorph liver oil be added once a day to the feeding formula. In the following weeks the baby "did not take the bottle well," leaving about half of it. A record of the weight

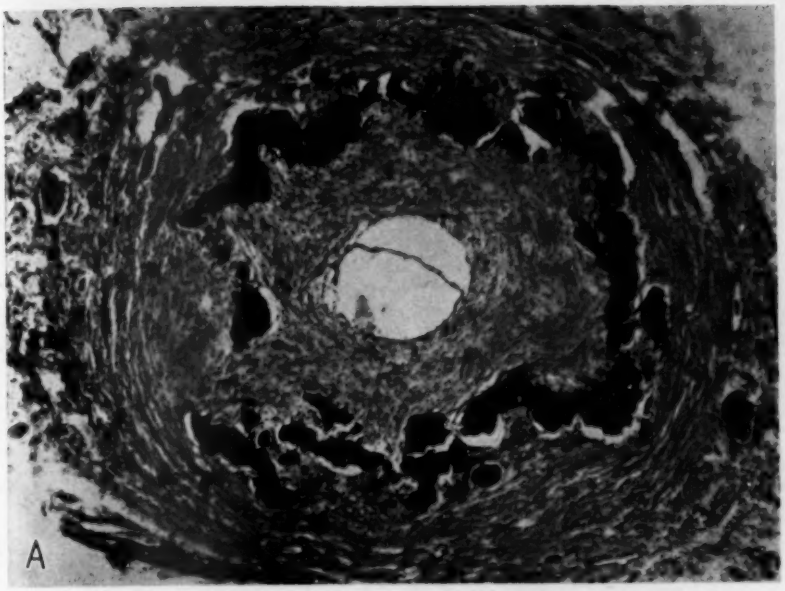
From the Department of Pathology, Ellis Hospital.

could not be obtained. The week before admission the mother noticed that the baby seemed to lose weight. It would lie for hours in the crib without making a noise, as if it were too weak to move or cry. On September 7 the baby was taken to the office of a local physician. Immediate hospitalization was advised.

When admitted to the hospital the next day the infant was cyanotic and undernourished, and the turgor of tissue was poor. The anterior fontanel was soft. The extremities were cold and clammy; the respirations, shallow and labored. The temperature was 97.4 F. The infant was placed in an oxygen tent and a 5 per cent dextrose solution administered by clysis. Its color did not improve, and death occurred three hours later.

Necropsy (ten hours after death).—The body was that of an emaciated infant girl of gracile build, 56 cm. long and weighing 3.4 Kg. The skin of the face and the finger nails were deeply cyanotic. There were no external signs of congenital syphilis or rickets. The pleural cavities contained no free fluid; the lungs were crepitant, although of firmer consistency than normal. The thymus weighed 12 Gm. and appeared normal. The pericardial cavity contained about 15 cc. of a straw-colored fluid. The heart weighed 35 Gm. The right ventricle appeared dilated. The coronary arteries were prominent, rigid and tortuous. They cut with gritty resistance, and their lumens were scarcely visible. The epicardium was smooth; the myocardium, pale and of less firm consistency than normal. The valves and the endocardium showed no lesions. The foramen ovale was closed. The aorta, the pulmonary arteries and the carotid arteries appeared normal on gross examination. The liver extended 6 cm. below the xiphoid process and on section revealed a mottled light and dark brown cut surface. The gastrointestinal tract showed no anomalies. The spleen, the pancreas, the adrenal glands, the kidneys, the urinary bladder and the pelvic organs were normal on gross examination: likewise, the ribs, the vertebrae and the skull. The brain showed no lesions, and all the cerebral vessels were thin and delicate. The organs of the neck revealed no significant anomalies. The parathyroid glands occupied their normal position and were not enlarged. Unfortunately, the changes observed later microscopically, were not anticipated at the time of the autopsy and the vessels of the extremities were not examined.

Microscopic Examination.—Both coronary arteries and their main branches showed extensive changes, characterized by medial calcification, intimal proliferation and consequent narrowing of the lumens. The thickening of the wall was due to marked fibroblastic proliferation of the subendothelial layer of the intima. Immediately adjacent to it was a continuous ring of calcareous material, which stained deep bluish violet with hematoxylin-eosin. The calcareous ring varied greatly in thickness; in places it was thin (fig. 1 *A*), and in others it occupied nearly one half of the thickness of the wall. Calcification of the internal elastica was noted in some vessels, but the membrane still retained its acidophilic properties and could be identified as a thin line within the calcium strands. The outer layer of the media was thin. In some vessels its muscle fibers were well outlined; in others, fragmented. The only noteworthy change in the adventitia was the presence of small focal collections of lymphocytes and eosinophils in one coronary artery. The innermost layer of the intima appeared to be normal. In the sections of the myocardium there were focal areas in which the muscle fibers were replaced by fibrous connective tissue. Sections of the arch of the aorta showed no significant changes. Lesions similar to those encountered in the coronary arteries were found in the arteries of the larynx, the thyroid gland, the mesentery, the pancreas, the adrenal glands and the kidneys. In the spleen, one artery sectioned



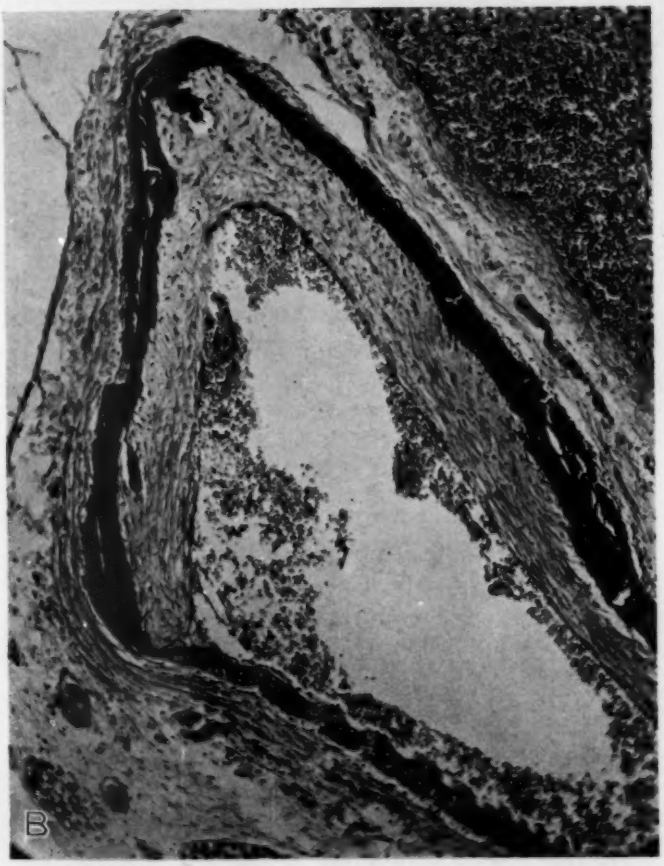


Fig. 1.—*A*, low power photomicrograph of the left coronary artery, showing calcium deposits in the media and fibroblastic proliferation of the intima. *B*, splenic artery. Note the abrupt line between the normal and the involved portion of the arterial wall.

*Summary of Data on Fifteen Infants in Whom Medial Calcification of Arteries Was Observed**

Author	Age	Sex	Arteries Involved †					Calcium Deposits in Other Organs	Other Findings	Factors Stated by Authors to Be Responsible for Arterial Calcification
			Aorta	Pulmonary Arteries	Coronary Arteries	Visceral Arteries	Arteries of Brain			
Durante [1]	17 days	?	+	+	?	?	?	0	Infection of navel, acute peritonitis	intrauterine infection
Bryant and White [2]	6 mo.	M	0	0	+	+	0	+	Congenital urethral dilatation, hydronephrosis	Renal disease
Surbeck [3]	3 days	M	+	0	+	+	0	+	Fibrinous pericarditis, splenomegaly	Sepsis, nephritis, "calcium gout"
Verocay [4]	5 mo.	F	0	0	+	+	?	0	Congenital syphilis	Congenital syphilis
Jaffé [5]	2 days	M	0	+	0	0	0	0	.....	Hydramnios, toxic factor of maternal origin
Hughes and Perry [6]	7 wk.	F	0	0	+	?	?	0		
Forrer [7]	3 mo.	M	0	0	+	+	?	0	Granuloma of navel, splenomegaly	Sepsis, dystrophic calcification
Iff [8]	1 day	M	+	+	+	+	0	0	Ascites	Hydramnios, incomplete development of "ground substance" of arteries
Oppenheimer [9] ‡	6 mo.	F	0	0	+	+	?	0		
Oppenheimer [9] ‡	4 mo.	F	0	0	+	+	?	0	Nephritis (?)	
Oppenheimer [9] ‡	11 days	F	+	+	?	?	?	+	Congenital malformation of genitalia	Infection of urinary tract
Ross and Williams [10]	.....	?	0	0	+	+	?	+	Bronchopneumonia	Hypervitaminosis D
Brown and Richter [11]	3 mo.	M	0	0	+	+	?	+	.....	Alteration in calcium-phosphorus metabolism, hypervitaminosis D(?)
Baggenstoss and Keith [12]	8 wk.	F	+	+	+	+	?	0	.....	Disturbance of calcium metabolism, abnormality of arterial wall
Field	10 wk.	F	0	0	+	+	0	0	.....	Physicochemical or toxic changes in the intercellular matrix of arterial wall

* The group does not include infants older than 12 months.
† An interrogation sign indicates that the arteries were not mentioned by the author.
‡ Oppenheimer reviewed 15,000 autopsy reports and found that arterial calcification had been noted in 3 infants.

See footnotes on next page

showed a continuous ring of calcium in the media. In another artery the calcification of the media and the proliferation of the intima were present in about one half of the circumference of the vessel, while the other half appeared entirely normal. There was an abrupt line between the normal and the involved portion of the wall (fig. 1 *B*). A small artery in the pericapsular fat of the adrenal gland revealed large irregular deposits of calcium in the media and the intima separated by fibrous-like tissue. Only a small lumen remained. In sections of both lungs edema of the septums was noted. The branches of the pulmonary arteries showed scattered longitudinal streaks of calcareous deposits in the intercellular matrix along the surface of the elastic fibers of the media.

There was a moderate increase of the fat content of the cells of the liver, but no other abnormality was noted.

The parenchymal cells of the parathyroid glands were normal in size, shape and staining qualities and in relation to each other.

The basilar artery and the arteries of the circle of Willis were normal.

COMMENT

Fourteen reports of arteriosclerosis occurring in infants are found in the literature. A review of the cases shows that from the point of view of the morphology the lesions are similar if not identical in all. The inner and the middle third of the media are the site of predilection for the deposition of calcium, where it is intimately related to the internal elastic lamina and to the interstitial substance adjacent to this. Associated with the calcification of the media is fibroblastic proliferation of the intima with consequent narrowing or obliteration of the lumen. Specific degenerative changes (lipoid deposits, hyalinization) are nowhere encountered in the intima.

The topographic distribution of the lesions of the arterial tree varies from case to case, but, generally speaking, the medium-sized and small arteries are those most frequently affected. The aorta and the pulmonary artery were involved in 5 of the reported cases. It seems worth while to mention that the infants showing involvement of the great vessels were young (see table).

FOOTNOTES TO TABLE

1. Durante, G.: Bull. et mém. Soc. anat. de Paris **74**:97, 1899.
2. Bryant, J. H., and White, W. H.: Guy's Hosp. Rep. **55**:17, 1901.
3. Surbeck, K.: Zentralbl. f. allg. Path. u. path. Anat. **28**:25, 1917.
4. Verocay, J.: Frankfurt. Ztschr. f. Path. **24**:109, 1920.
5. Jaffé, R.: Frankfurt. Ztschr. f. Path. **15**:118, 1914.
6. Hughes, F. W. T., and Perry, C. B.: Bristol Med.-Chir. J. **46**:219, 1929.
7. Forrer, H.: Ausgedehnte Gefässverkalkung im frühen Kindesalter, Inaug. Dissert., Zurich, J. H. Meier, 1930.
8. Iff, M.: Virchows Arch. f. path. Anat. **281**:377, 1931.
9. Oppenheimer, E. H.: Bull. Johns Hopkins Hosp. **63**:261, 1938.
10. Ross, S. G., and Williams, W. E.: Am. J. Dis. Child. **58**:1142, 1939.
11. Brown, C. F., and Richter, I. M.: Arch. Path. **31**:449, 1941.
12. Baggenstoss, A. H., and Keith, H. M.: J. Pediat. **18**:95, 1941.

The vast majority of investigators have enumerated many different sites of sclerotic lesions but have failed to mention the cerebral vessels. The few authors who have described the cerebral arteries have pointed out the absence of arteriosclerosis.

In the present case the aorta and the pulmonary artery, as well as the vessels of the brain, were free of lesions. It seemed of interest to determine the exact point in the vascular tree where the earliest changes would first make their appearance. The common carotid artery provided this opportunity. The proximal end of this artery showed focal change in the intercellular substance of the media, characterized by an increase in its volume and a decrease in the intensity of its staining qualities. A section through the distal end of the same artery revealed more advanced changes, such as fine granular blue-stained deposits formed along the medial surface of the internal elastic membrane (fig. 2*A*). The thyroid and laryngeal arteries, on the other hand, presented extensive plaques of calcium in the media, proliferation of the intima and nearly complete obliteration of their lumens (fig. 2*B*).

As to the clinical signs of the disease under discussion, there are insufficient data available to suggest a symptom complex. In several reported cases, as well as in the present case, failure of general health without obvious cause, persistent anorexia, fretfulness and apathy were the prominent features.

There has been general uncertainty among the authors as to the etiologic factors and the causative mechanism of the disease. However, in an analysis of the cases reported the following facts appear to be significant. Infection, acute or chronic, preceded the death of the infant in 5 reported cases, and was attributed by the authors as the cause of the lesions found.

The theory of an infectious genesis of arteriosclerosis, championed originally by Virchow and supported later, in a modified form, by Klotz [13] and others, assumes that bacteriotoxins present in the blood injure the intima and prepare it for the subsequent atheromatous change by increasing its permeability. An infectious origin (diphtheria, scarlet fever, measles) of intimal and medial degeneration of the arteries of children was advocated by Wiesel.[14] Karsner [15] too has felt that among various factors which operate in the production of arteriosclerosis infectious disease plays a prominent part. However, the etiologic significance of infectious diseases has been disputed by other

13. Klotz, O.: Brit. M. J. **22**:1767, 1906.

14. Wiesel, J.: Wien. klin. Wchnschr. **56**:1421, 1926.

15. Karsner, H. T.: Human Pathology, Philadelphia, J. B. Lippincott Company, 1942, p. 385.

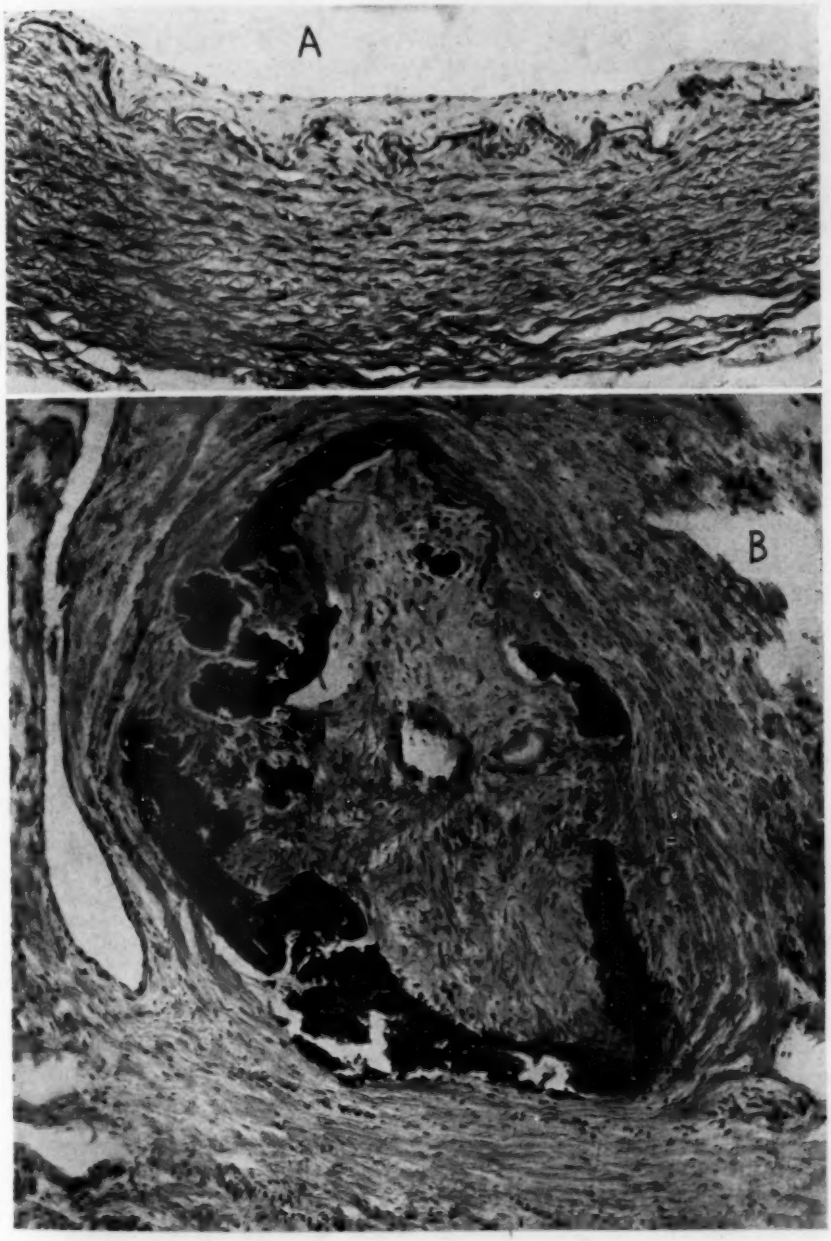


Fig. 2.—*A*, high power photomicrograph of the common carotid artery, showing early change. Note the incrustation of calcium along the internal elastic membrane. *B*, low power photomicrograph of the thyroid artery.

investigators.[16] MacCallum [17] concluded from an analysis of the available clinical, pathologic and experimental evidence that there is little in favor of the idea that acute and chronic infections play a major part in the genesis of arteriosclerosis. As far as the group of infants under discussion is concerned, the authors have pointed out that the earliest lesions detected are in the media and not in the intima as would be expected according to the theory championed by Virchow.

There is no doubt that the arterial calcification observed by Iff in a 1 day old premature infant was acquired in utero. This author insisted that primary arterial damage preceded the calcific change. Furthermore, he maintained that incomplete development of the "ground substance" of the arteries is responsible for the lesions found. This so-called ground substance was much discussed in the German medical literature around 1920. According to Hueck,[18] it is a loose, undifferentiated, syncytium-like tissue, which is the "mother substance" of the arterial wall and a precursor of collagen and elastic tissue and may be found in the arterial wall between the fibers of the latter substances.

Prior to Hueck, Björling [19] described loose connective tissue fibers found in the walls of arteries, which he differentiated from collagen and elastic tissue by their metachromatism. Those fibers stained red with Unna's polychrome–methylene blue, in contrast to the blue-staining collagen and elastic fibers. He was unable to demonstrate this substance anywhere in the body aside from the arterial wall. In arteriosclerosis and syphilis of the aorta he observed an increase of this substance in proportion to regression of elastic and muscle fibers. Schultz [20] suggested for demonstration of this substance, which he called chromotropic ground substance, cresyl violet (ground substance deep red, muscle fibers pale blue, elastic fibers purple). He was able to demonstrate the chromotropic substance in the alveolar walls of the lungs, in the umbilical cord and the placenta and in the capsule of the glomerulus. According to Schultz, this substance has a special affinity for calcium and lipids, probably because of its high content of chondroitin-sulfuric acid. Sslowjew,[21] in a detailed report on the intercellular substance of the arterial wall, stated that it is most abundant in the inner sheaths of the media in arteries of the elastic type. But he was unable to demonstrate it in the walls of the smaller arteries.

16. Zinserling, W. D.: Zentralbl. f. allg. Path. u. path. Anat. **24**:627, 1913. Lange, F.: Virchows Arch. f. path. Anat. **248**:463, 1924.

17. MacCallum, W. G.: Acute and Chronic Infections as Etiological Factors, in Cowdry, E. V.: Arteriosclerosis, New York, The Macmillan Company, 1933, p. 355.

18. Hueck, W.: München. med. Wchnschr. **67**:535 and 573, 1920.

19. Bjorling, E.: Virchows Arch. f. path. Anat. **205**:71, 1911.

20. Schultz, A.: Virchows Arch. f. path. Anat. **239**:415, 1922.

21. Ssolowjew, A.: Virchows Arch. f. path. Anat. **241**:1, 1923.

Iff expressed the belief that the toxic agent which caused hydramnios in the mother is also responsible for the incomplete maturation of the ground substance in the prematurely born, 1 day old infant, referred to. It is noteworthy that a severe grade of hydramnios was also present in a 2 day old infant with calcification of the pulmonary artery examined by Jaffé.[5]

The fact that calcium deposits were found in various organs aside from the arteries led some authors to the assumption that a disturbance in calcium metabolism resulting in inability of the serum to retain all its calcium in solution is responsible for the lesions found.

The present knowledge of calcium metabolism is admittedly incomplete. There is more calcium in the serum at all times than can be accounted for by the laws of simple solution. Barr [22] stated that only 25 per cent of the serum calcium can be accounted for by the laws of simple solution. At least two other factors, it is believed, are active to maintain the remainder of the calcium in serum. The more important of these is the hormone of the parathyroid gland, which holds about 50 per cent of the normal serum calcium in solution. In some way the action of vitamin D is related to this fraction of the total serum calcium. Another factor is the combining effect of the serum proteins, which holds about 30 per cent of the normal serum calcium in solution.

It has been shown by Ham and Portuondo [23] in experimental work on animals that abnormal calcifications of tissue may occur with total blood calcium values that are not elevated, and, conversely, the serum calcium can remain high for a long time without producing pathologic calcification.

The serum calcium was not determined in the present case or in the other discussed cases. However, from the clinical data available one may assume that alteration of the blood calcium-phosphorus balance was present in the group of infants showing advanced renal damage (see table).

Since Mitchell [24] published his extensive study and thorough review of the literature on renal rickets, this condition has been more frequently reported in the American literature. Mitchell has offered the hypothesis that when there is renal retention of phosphates the waste phosphates are excreted into the intestinal tract, where they combine with the calcium of the food and so block absorption of the latter. The child suffers true calcium starvation, followed by depletion of the calcium of the bones and possibly other tissues. Mitchell reviewed about 190 cases, and in 22 the necropsy notes included the statement that the aorta and other arteries were thickened and sclerotic. An exact mor-

22. Barr, D. P.: Physiol. Rev. **12**:593, 1932.
23. Ham, A. W., and Portuondo, B. C.: Arch. Path. **16**:1, 1933.
24. Mitchell: Am. J. Dis. Child. **40**:130, 1930.

phologic description of the lesions was not given. Lightwood [25] observed renal dwarfism in a child 2 years and 3 months old. The anatomic character of the arterial lesions in his patient appears identical with that of the lesions found in the group of infants under discussion.

Another condition associated with disturbance of calcium and phosphorus balance and mentioned by several authors as the cause of arterial calcifications is hypervitaminosis D. Observations in man in regard to the arteriosclerotic action of massive doses of vitamin D are relatively scant. Some of the reported cases are of accidental nature; others are of experimental character. The diagnosis of overdosage of viosterol in the cases reported by Eisler [26] and Thomason [27] was based solely on abnormal vascular calcification detected by roentgenographic examination. Autopsies were made in the case reported by Putschar [28] and 2 cases reported by Thatcher.[29] In each of these cases extensive calcium deposits were found in the kidney, but none in the arteries. Ross and Williams [10] recorded toxic symptoms from large doses of vitamin D in 4 infants. In this group there were two deaths; one postmortem examination was performed, which revealed widespread calcification of the arteries (inner third of the media) as well as calcium deposits in the kidneys and the myocardium.

Wolf [30] attempted to determine the toxic dose of activated vitamin D by administering massive doses to a 4 month old infant afflicted with spina bifida and meningomyelocele; 300,000 U. S. P. units of vitamin D was given daily for two weeks. The patient died following a diagnostic cisternal puncture six weeks after completion of the therapy. Postmortem examination showed deposits of calcium in the renal tubules but none in other organs, including arteries.

Much experimental work has been done to determine the toxic effect of excessive doses of irradiated ergosterol on laboratory animals. It has been shown repeatedly [31] that massive doses produce medial calcification and intimal proliferation in the walls of the arteries, lesions which appear essentially similar to those found in the group of infants under discussion.

Vanderveer,[31c] in his report on arteriosclerotic lesions produced in rabbits by excessive doses of vitamin D, pointed out that calcium

25. Lightwood, R.: Arch. Dis. Childhood **7**:193, 1932.

26. Eisler, F.: Klin. Wchnschr. **9**:1846, 1930.

27. Thomason, F.: Acta med. Scandinav. **93**:505, 1937.

28. Putschar, W.: Klin. Wchnschr. **8**:858, 1929.

29. Thatcher, L.: Edinburgh M. J. **38**:456, 1931; Lancet **1**:20, 1936.

30. Wolf, I. J.: J. Pediat. **22**:707, 1943.

31. (*a*) Pallske, G.: Klin. Wchnschr. **11**:1060, 1932. (*b*) Schiff, A.: Virchows Arch. f. path. Anat. **278**:62, 1930. (*c*) Vanderveer, H. L.: Arch. Path. **12**:941, 1931.

is first deposited in the intercellular matrix of the media. This author stated: "It is surprising what an accumulation of calcareous material is first deposited over and about the elastic fibers and muscle cells before either show evidence of marked degeneration."

The mechanism by which the calcifications in hypervitaminosis D are produced is still an unsolved problem. Some authors (Vanderveer,[31c] Wolbach and Bessey [32]) have felt definitely that cellular damage occurs first and that calcium is then deposited in the injured areas. Ham,[33] on the other hand, maintained that pathologic calcifications are not preceded by cellular damage, pointing out that he was unable to demonstrate changes in cells twenty-four hours after the drug had been administered to rats and yet after forty-eight hours massive calcification occurred. The possibility has been suggested that vitamin D promotes calcification in the body through a stimulating action on the parathyroid glands. It is notable that the sites at which calcium is deposited in tissues of animals treated with parathyroid extract (Leaner [34]; Hueper [35]) are similar to those at which it is deposited in animals as a result of hypervitaminosis D.

McJunkin, Tweedy and Breuhaus [36] concluded from their experimental work with parathyroid preparations that the lesions observed in tissues were caused not by direct action of calcium but by a disturbance of the calcium content of the cells altering the calcium balance between these cells and the tissue fluids. Ham [33] suggested that the withdrawal of calcium from the tissue cells during the upswing of the serum calcium curve in hypervitaminosis D should be considered as a possible cause of the injury of the cells.

It may be pointed out, however, that the arterial lesions found in animals treated with massive doses of ergosterol or with parathyroid preparations are similar to those produced experimentally by administration of diphtheria toxin, epinephrine hydrochloride, barium chloride and digitalin. The mode of action of the latter agents is difficult to correlate with calcium metabolism.

Considering the lesions in the patient from the point of view of etiology, one has to admit that the cause is obscure. The healthy appearance of the organs excludes an infectious origin. There is no evidence to indicate an alteration of blood calcium-phosphorus balance, as the parathyroid glands were histologically normal and the kidneys

32. Wolbach, S. B., and Bessey, O. A.: Physiol. Rev. **22**:233, 1942.

33. Ham, A. W.: Arch. Path. **14**:614, 1932.

34. Leaner, A.: J. Lab. & Clin. Med. **14**:921, 1929.

35. Hueper, W. C.: Arch. Path. **3**:14, 1927; J. Lab. & Clin. Med. **19**:1293, 1934.

36. McJunkin, F. A.; Tweedy, W. R., and Breuhaus, H. C.: Arch. Path. **14**: 649, 1932.

showed no inflammatory or degenerative changes. The possibility of hypervitaminosis D cannot be entirely excluded. The amount of vitamin D given to the infant, if administered correctly, is in no sense comparable to the doses given experimentally to produce similar lesions in animals.

The fact that the intercellular matrix (ground substance) of the arterial wall was the site of the primary lesions in the clinical group of infants, as well as in experimental animals in which arteriosclerosis was produced by various agents mentioned in a foregoing paragraph, makes one firmly believe that specific changes in the intercellular substance are responsible for the ensuing calcification. One may assume that this intercellular matrix is a system of colloidal nature which is capable of functioning as an organ which can become diseased. Physicochemical reactions followed by changes in the colloidal state of this substance, or toxic injury of it, may favor formation of calcium deposits. The cause of injury may be the withdrawing of calcium from tissue cells in renal rickets (calcium starvation) or in hypervitaminosis D during the upswing of the serum calcium curve (Ham). Hypercalcemia as such does not deserve the importance ascribed to it, for calcium, as is well known, tends to precipitate in degenerated or nonviable tissue (e. g., old tuberculous lesions or infarcts of placenta).

That infectious diseases preceded the deaths of 5 infants cannot be overlooked. The mechanism by which the bacteria or their toxins injure the vascular wall is obscure, as no cellular infiltration occurred in or about the lesions.

The cause of the arterial changes found in the patient remains undetermined.

SUMMARY

Extensive medial calcification of arteries was observed in an infant 10 weeks old. The arterial calcification noted in this and other infants in whom similar findings were made, as shown by a review of the literature, differs from the arteriosclerosis of adults inasmuch as no specific degenerative changes of the intima preceded the medial calcification. The primary site of the calcium deposit appears to be the intercellular substance of the media, which suggests that an injury of this substance is the cause of the calcification.

The arterial lesions found in the aforementioned group of infants resemble the arteriosclerosis experimentally produced in animals by administration of diphtheria toxin, epinephrine hydrochloride, barium chloride, digitalin, parathyroid extract and massive doses of vitamin D.

Mr. E. G. Moore of the Albany Medical College supplied the photomicrographs.

PATHOLOGIC CHANGES RESULTING FROM THE ADMINISTRATION OF STREPTOMYCIN

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RAHWAY, N. J.
AND
HARRISON S. MARTLAND, M.D.
NEWARK, N. J.

SINCE streptomycin was isolated from Actinomyces (Streptomyces) griseus by Schatz, Bugie and Waksman [1] in 1944, many reports have appeared on the experimental and clinical chemotherapeutic efficacy of this antibiotic agent. The literature concerning this drug has been surveyed recently by Waksman and Schatz.[2]

In view of the definite antibacterial action of streptomycin against numerous gram-negative organisms as well as against certain gram-positive organisms and Mycobacterium tuberculosis, the widespread use of this substance is to be anticipated. Studies on the acute lethal toxicity of streptomycin indicate that its toxicity is of a low order.[3] Preliminary findings on the pathologic effects of streptomycin have been reported from this laboratory as part of a general pharmacologic investigation of the drug (Molitor and co-workers [3b]). The present communication is an extended report on the pathologic changes produced in laboratory animals by the administration of streptomycin for brief and for prolonged periods.

MATERIALS AND METHODS

Forty-two monkeys, 11 dogs, 350 rats, 100 mice, 10 chickens and 154 guinea pigs were used in these experiments. All these animal species were housed in

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The work described in this paper was done in part under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development of the National Research Council and the Merck Institute for Therapeutic Research, Rahway, N. J.

1. Schatz, A.; Bugie, E., and Waksman, S. A.: Proc. Soc. Exper. Biol. & Med. **55**:66, 1944.

2. Waksman, S. A., and Schatz, A.: J. Am. Pharm. A. (Sc. Ed.) **34**:273, 1945.

3. (*a*) Robinson, H. J.; Smith, D. G., and Graessle, O. E.: Proc. Soc. Exper. Biol. & Med. **57**:226, 1944. (*b*) Molitor, H.; Graessle, O. E.; Kuna, S.; Mushett, C. W., and Silber, R. H.: J. Pharmacol. & Exper. Therap. **86**:151, 1945.

air-conditioned quarters at a temperature of 75 F. and a relative humidity of 50 per cent. The monkeys, Macacus rhesus, weighing between 3 and 6 Kg., were fed a mixed diet consisting of Purina dog chow, fresh fruits and vegetables. The dogs, mongrels weighing 8 to 11 Kg., were given a mixture of Gaines dog meal, fresh horse meat and milk. Nutritionally complete diets were also provided for the rats (Wistar strain), mice (CFW strain), chickens and guinea pigs.

Most of the studies were carried out with streptomycin hydrochloride concentrates[4] varying in potency from 250 to 400 micrograms of streptomycin base per milligram of solids.[5] Highly purified streptomycin, with a potency of 800 micrograms streptomycin base per milligram of solids, was used in only a few experiments. The streptomycin was administered subcutaneously, intramuscularly or intravenously in an aqueous neutral solution in doses of 10 to 200 mg. per kilogram. Many of the animals received the drug daily for periods of about five days, but in several experiments the dosing period was extended to several months or more.

Samples of urine were collected alternately with and without toluene. The erythrocyte, leukocyte, differential and platelet counts, the sedimentation rate, the hematocrit value, the icteric index and the prothrombin level (on both 100 and 12.5 per cent plasma) were determined on blood samples drawn at frequent intervals. Tissues for microscopic examination were fixed in neutral solution of formaldehyde U.S.P. (1:10) and also in one or more of the following: Helly's fluid,[6] formaldehyde-alcohol (4 per cent formaldehyde in absolute ethyl alcohol), absolute acetone and Regaud's solution. The stains employed were hematoxylin-eosin, sudan IV, Best's glycogen stain and others as indicated. Since the most complete studies were made in monkeys and dogs, the major portion of this communication will deal with these species.

PATHOLOGIC EFFECTS ON THE LIVER AND THE KIDNEY

Macroscopic Changes.—The internal organs of most of the species treated with streptomycin appeared normal, but pale areas were noted in the livers and the kidneys of dogs and monkeys. The median lobe of the liver of 1 dog showed tan areas suggestive of necrosis. Occasionally in dogs the gallbladder was distended, the lower part of the jejunum and the ileum were bile stained and the stools appeared green and tarry.

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4. All streptomycin used in this study was supplied by the Research Laboratories of Merck & Co., Inc.

5. The potency of a streptomycin concentrate was formerly expressed in terms of units, one unit being that quantity which will just inhibit a given strain of Escherichia coli in 1 cc. of nutrient medium. As recommended by the Food and Drug Administration, the potency of streptomycin is now designated in terms of its equivalent in weight of pure streptomycin base. One microgram of streptomycin base is equivalent to 1 unit. In this paper all doses are expressed in terms of weight of streptomycin base administered; the weight does not refer to the actual weight of the streptomycin concentrate.

6. Potassium dichromate, 2.5 Gm.; mercuric chloride, 5 Gm.; sodium sulfate, 1 Gm.; water, 100 cc., and solution of formaldehyde, U.S.P., 5 cc., added before use to each 100 cc. of fluid. This is a modified form of Zenker's fluid.

Microscopic Changes.—(*a*) Liver: The cytoplasm of the hepatic cells appeared vacuolated and sparsely granular in dogs and monkeys treated with streptomycin in doses of 25 mg. per kilogram or more. Sudan IV stain revealed moderate to marked fatty metamorphosis, with the fat present in the cord cells or the Kupffer cells in fine to moderate-sized globules (*A*, fig. 1). When present in large quantities the fat showed uniform distribution throughout the lobules (*B*, fig. 1); pericentral concentration was noted when a lesser amount was present. Glycogen was equivalent to or greater than that found in control animals. Like the fat, its lobular distribution was uniform (*C*, fig. 1). No inverse relationship existed between the amount of fat and the amount of glycogen present. In fact, some of the livers containing the greatest quantity of glycogen also showed the most pronounced fatty metamorphosis.

Multiple small foci of necrosis, with round cell and occasionally polymorphonuclear cell infiltration (*A*, fig. 2), were seen in the livers of a few dogs. Fibroblastic proliferation was seen also in some areas. Fine yellow granules were present in the cord cells, particularly around the bile canaliculi. Iron-positive pigment deposits were seen in the centrolobular areas.

In an experiment designed to permit study of the genesis and the course of the fatty metamorphosis in the liver, monkeys were given five daily intravenous injections of 25 mg. per kilogram and killed in pairs at intervals of one, ten, thirty and sixty days after the last injection. A small amount of lipoid material was present in liver sections after one day. On the tenth day an increased amount was present (*B*, fig. 1). Little fat was seen after thirty days (*D*, fig. 1), and by sixty days it was absent. These data indicate that the fatty metamorphosis resulting from the administration of streptomycin is a reversible phenomenon and does not represent progressive degeneration.

Monkeys treated by daily subcutaneous injection of 25 mg. per kilogram for sixty-six consecutive days did not present pathologic changes any more impressive than those observed in monkeys given the same dose of the drug intravenously for a period of only five days.

The fatty change occurred in animals treated with highly purified streptomycin (800 micrograms streptomycin base per milligram of solids) as well as with less pure concentrates.

No gross or microscopic changes which could be attributed to streptomycin were observed in the livers of rats (100 mg. per kilogram daily, subcutaneously, for seventy-two days), guinea pigs (60 mg. per kilogram daily, subcutaneously, for seventy days), mice (200 mg. per kilogram daily, subcutaneously, for ten days) or chickens (100 mg. per kilogram daily, subcutaneously, for fourteen days).

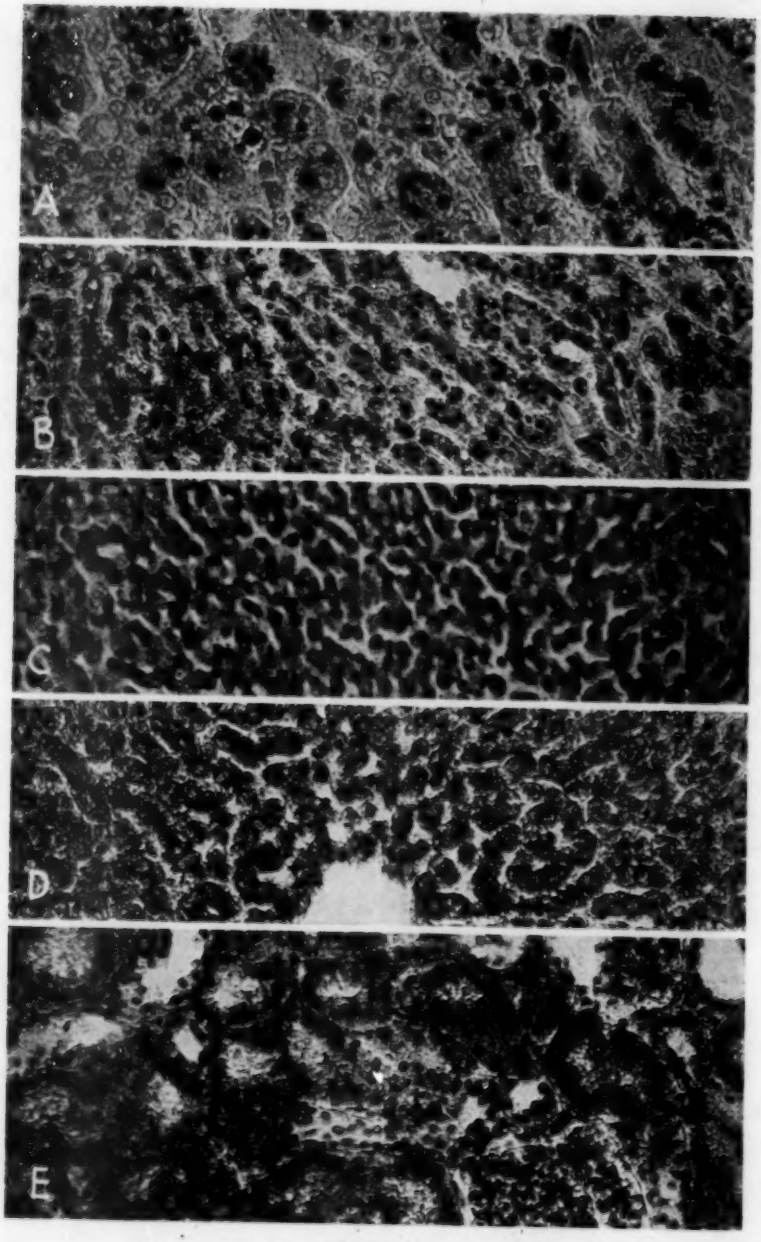

Figure 1

(See legend on opposite page)

(*b*) Kidneys: Examination was made of the sediment of centrifuged urine of monkeys, dogs and rats. In the urine from a few monkeys given several large doses of streptomycin or smaller doses for prolonged periods, an occasional cast, epithelial cell or blood cell was seen, but the presence of these formed elements in the urine was never consistent or great in extent and can be considered to be of little significance, particularly since the urine of control animals at times showed a similar picture. The urine of rats treated by parenteral injection of 100 mg. per kilogram for approximately two and one-half months showed no changes. That of dogs receiving 50 or 100 mg. per kilogram daily showed, within a few weeks, casts, epithelial cells and leukocytes. An occasional erythrocyte was seen in the urine of a few animals. We have reported the occurrence of albuminuria in several of these animals.[3b] A report of the biochemical studies conducted on these animals will be published elsewhere.[7]

On histologic examination, pale eosinophilic granular detritus was present in the glomerular spaces and the tubules of the kidneys of several monkeys in which proteinuria had developed after treatment for five to ten days with 100 or 200 mg. per kilogram. No casts, however, were present in the tubules. Several kidneys had a slight degree of fatty degeneration in the convoluted tubules (*E*, fig. 1). In the kidney, as in the liver the fatty metamorphosis was found to be reversible. Thus in monkeys killed ten days after the last of five daily intravenous injections of 25 mg. per kilogram fat was present in the renal parenchyma, but none was seen in animals killed thirty or sixty days after such treatment. The fine lipoid globules occupied the basal portion of the epithelial cells or the interstitial tissue of the collecting tubules. Rarely, brownish refractile granules were present also in the epithelium

7. Silber, R. H.; Porter, C., and Clark, I.: To be published.

EXPLANATION OF FIGURE 1.

Pathologic changes in tissues of monkeys treated by intravenous injections of streptomycin—25 mg. per kilogram daily for five days.

A, fatty metamorphosis of liver. Lipoid material is present in fine to moderately large globules. The animal was killed ten days after the last injection. Sudan IV stain; × 300.

B, lipoid material uniformly distributed throughout a hepatic lobule. Low power magnification of *A* (× 130).

C, liver containing abundant, evenly disposed glycogen deposits. The animal was killed ten days after the last injection. Best's carmine stain; × 130.

D, liver of a monkey used in an experiment to demonstrate reversibility of fatty metamorphosis. Only a small amount of lipoid material is present in the pericentral area. The animal was killed thirty days after the last injection. Sudan IV stain; × 130.

E, fatty metamorphosis of kidney. Tubules containing lipoid globules are present between the two glomeruli and in the lower portion. The animal was killed ten days after the last injection. Sudan IV stain; × 130.

of the convoluted tubules. Staining of renal tissue for glycogen revealed none, and examination of unstained frozen sections with Nicol prisms failed to reveal doubly refractile droplets of cholesterol esters.

Focal necrosis (*B*, fig. 2), sometimes accompanied by epithelial desquamation, was observed in the convoluted tubules of the kidneys of 1 dog, and patchy areas of round cell infiltration were seen in the renal

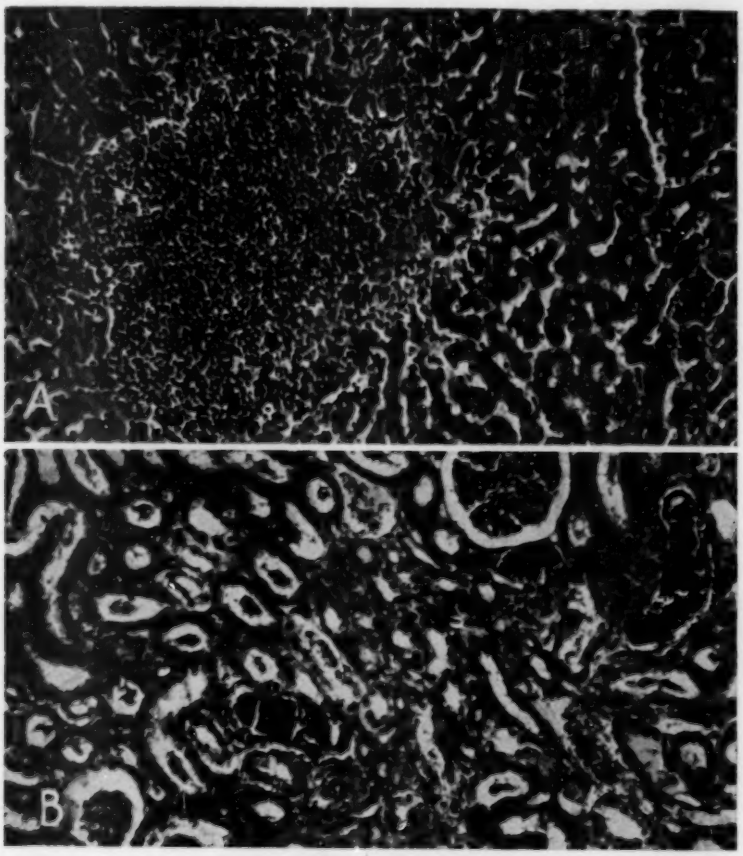

Fig. 2.—Pathologic changes in tissues of dogs treated by intramuscular injection of streptomycin—100 mg. per kilogram daily for twenty consecutive days. The animals were killed fifteen days after the last injection.

A, focal necrosis of liver. Note also small aggregations of lymphocytes. Hematoxylin and eosin; × 150.

B, focal necrosis with calcification in the convoluted tubules of the kidney. The glomeruli appear normal. Hematoxylin and eosin; × 150.

tissue of 2 dogs treated by subcutaneous injection of 100 mg. per kilogram per day for twenty days. Basophilic granular material (calcium) was frequently present at the necrotic sites. Hyaline casts and

occasionally iron-positive casts occurred in the collecting tubules. Much stainable lipoid material was evident in the cells of the convoluted segment of the nephron and the limbs of Henle's loop; less was seen in the epithelium of the collecting tubules.

The presence of granular albuminous detritus in the glomerular spaces and tubules of the kidneys of some monkeys treated with streptomycin indicated increased permeability of the glomerular capillary membranes. That this change is a reversible one is suggested by the fact that certain monkeys which had shown clinical evidence of proteinuria and later recovered did not have granular detritus present in the nephrons.

None of the other species given injections of streptomycin showed pathologic changes in the kidneys.

TOXIC EFFECTS ON THE NERVOUS SYSTEM

Two dogs which had received daily subcutaneous injections of 100 mg. per kilogram of a streptomycin concentrate (potency, 250 mg. streptomycin base per milligram of solids) for twenty days were killed fifteen days after the last injection. Both animals had shown disturbances of equilibrium during the latter part of the dosing period. They were disposed to walk with a wavering gait in wide circles, with the head kept to one side. An impairment of auditory acuity was suggested by a failure of the dogs to respond normally to an unexpected loud sound. At the time of autopsy, several weeks after this condition was first observed, no improvement was noted in either animal. One of 3 dogs treated with 50 mg. per kilogram exhibited disturbances of equilibrium and of gait similar to, but milder than, those seen in the animals which had received 100 mg. per kilogram. The symptoms appeared after one and one-half months of treatment, but despite continued treatment this animal recovered several weeks later. Two dogs treated by subcutaneous injection for twenty days with samples of streptomycin of relatively high potency (640 to 710 micrograms of streptomycin base per milligram of solids) at a dose level of 100 mg. per kilogram failed to show neurotoxic symptoms.

In view of the equilibrial and possible auditory disturbances found in some of these dogs, histologic studies were made on the cerebrum, the cerebellum and the medulla and also on the acoustic, brachial and sciatic nerves. No pathologic alterations were observed in these tissues stained with hematoxylin-eosin, Bodian's stain and the Pal-Weigert stain.

It is believed, therefore, that the toxic manifestations exhibited by these animals may be due to degenerative changes in the vestibular and auditory end organs. To elucidate this possibility histologic studies

of temporal bones are being conducted in collaboration with Dr. E. P. Fowler Jr.[8] It is also possible that there may be some direct effect on the cerebellum or other nervous tissues which will require further study by more extensive neuropathologic methods.

EFFECTS ON THE BLOOD AND THE BLOOD-FORMING ORGANS

Complete and frequently determined blood values revealed as the only significant change slight normocytic anemia. This was usually observed after several daily injections of 50 mg. per kilogram or more in the monkey or after one week of daily injections of 100 mg. per kilogram in dogs. The anemia was transient, the cell counts returning to normal shortly after withholding of the drug. It persisted in 2 dogs during a period of reduced intake of food. Occasionally a slight increase in erythrocyte sedimentation rate occurred, due probably to the state of anemia. No significant changes in total or in differential leukocyte counts, platelet counts, prothrombin levels or icteric indexes were observed at any time.

Slight anemia was seen in rats receiving subcutaneous injections at a dose level of 100 mg. per kilogram of a low potency streptomycin concentrate (60 micrograms of streptomycin base per milligram of solids). When this was followed with samples of higher potency, the red cell count and the hemoglobin level returned to normal, and no further changes were observed in the blood picture, although treatment was continued for a total of seventy-two days.

Histologic examination of the femoral marrow of these monkeys, dogs and rats revealed no pertinent changes. In some of the animals the spleen showed an increased amount of hemosiderin, but the structure was normal.

The occurrence of anemia following streptomycin therapy would not appear to be of serious consequence, since the anemia is transient and no involvement of the marrow is indicated. In the clinical investigation of streptomycin, Zintel and co-workers,[9] Heilman and co-workers[10] and Foshay[11] observed no significant changes in the blood of their patients.

LOCAL EFFECTS AT SITES OF ADMINISTRATION

Muscle and Skin.—At autopsy the most striking gross effects noted were lesions occurring at the sites of injection. Small areas of

8. Fowler, E. P., Jr., department of otolaryngology, College of Physicians and Surgeons, Columbia University, New York.

9. Zintel, H. A.; Flippin, H. F.; Nichols, A. C.; Wiley, M. M., and Rhoads, J. E.: Am. J. M. Sc. **210**:421, 1945

10. Heilman, D. H.; Heilman, F. R.; Hinshaw, H. C.; Nichols, D. R., and Herrell, W. E.: Am. J. M. Sc. **210**:578, 1945.

11. Foshay, L.: J. A. M. A. **130**:393, 1946.

necrosis were seen in muscles in which as little as 10 mg. per kilogram had been injected. Dry scabs and occasionally ulcers of the epidermis were present in many animals treated by subcutaneous injection. The degree of local damage showed a direct relation to the potency of the material used. Thus, at a level of 100 mg. per kilogram no apparent effect resulted from samples with a potency of 225 to 400 micrograms of streptomycin base per milligram of solids, whereas a low potency preparation (30 micrograms per milligram) was responsible for the production of many open ulcers and scabs. Only a few small dry scabs were seen in the monkeys which received streptomycin of intermediate potency (135 micrograms per milligram).

Vein.—One monkey, weighing 4 Kg., was given by continuous intravenous infusion a total of 1.9 Gm. of streptomycin over a forty-two hour period. Microscopic examination of tisue from the cannulated area revealed an organizing thrombus in the vein and panphlebitis. Localized hemorrhage was also evident.

Pleural Cavities.—In anticipation of the use of streptomycin in the clinical therapy of empyema, experiments were conducted to determine the local effects produced by intrapleural injections of the drug. Solutions containing 1, 10 or 100 mg. of streptomycin per cubic centimeter were injected into the pleural cavities of rabbits at a dose of 1 cc. per kilogram. Two animals were used at each dose level. Four days after injection all animals had localized congestion and effusion of pleural fluid. Those which received the 100 mg. dose had small areas of hemorrhage in the pleural wall and fibrinous adhesions between the lung, the diaphragm and the pleural wall.

Gastrointestinal Tract.—Since streptomycin is absorbed to only a slight extent on oral administration,[12] one would not expect to find in the viscera of animals so treated pathologic changes comparable to those observed after parenteral dosage. Necropsy of mice dead from an oral dose of the drug revealed an excessive amount of fluid in the gastrointestinal tract and numerous small hemorrhagic lesions along the intestine. Similar findings were recorded when sodium chloride solutions of approximately the same osmotic pressure as the solutions of streptomycin were given. As suggested in a previous report,[3b] it appears that these effects on the gastrointestinal tract are due primarily to the osmotic concentrations of the streptomycin solutions employed, although the drug itself may have exerted a local irritating action in addition.

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12. Reimann, H. A.; Elias, W. F., and Price, A. H.: J. A. M. A. **128**:175, 1945. Stebbins, R. B.; Graessle, O. E., and Robinson, H. J.: Proc. Soc. Exper. Biol. & Med. **60**:68, 1945.

Mice which received streptomycin in their diet (25 mg. per gram of diet) for a period of four months showed no pertinent gross or microscopic alterations in their tissues.

CHANGES IN OTHER ORGANS

Buccal Lesions.—In 1 monkey given five daily subcutaneous injections of 100 mg. per kilogram a small suppurative ulcer developed at the tip of the tongue. This lesion was similar to but much less severe than those produced in both monkeys and dogs by streptotrichin.[13]

Adrenal Glands.—The adrenal gland of 1 monkey which had received a low potency preparation contained a calcified mass at the junction of cortex and medulla. This isolated observation may be of no significance.

Other Viscera.—No significant pathologic changes were seen in the following organs of any of the animals treated parenterally with streptomycin: heart, aorta, lungs, spleen, lymph nodes, pancreas, thyroid glands, adrenal glands, pituitary gland, testes, prostate, bladder, gastrointestianl tract, eyes.

COMMENT

Dogs and monkeys treated with streptomycin concentrates were found to have fatty metamorphosis of the liver and less often of the kidneys. Focal necrosis was seen infrequently in these organs. Long-continued dosage with streptomycin did not result in pathologic changes which were more marked than those seen after shorter periods of treatment. Evidence of pathologic damage in man following therapy with streptomycin is lacking.

However, neurotoxic signs similar to those observed in dogs in the present experiments have been seen in man. Thus Hinshaw and Feldman [14] described transient deafness and disturbances of the vestibular apparatus with marked vertigo in patients treated with large doses of streptomycin for prolonged periods. They are inclined to attribute this neurotoxic effect to involvement of the eighth nerve. More recently Lawrence [15] has observed cerebellar ataxia without apparent involvement of the eighth nerve or the labyrinth in a patient given a total dose of 14,000,000 units (14 Gm.) of streptomycin. The patient recovered within six weeks. No serious or uncontrollable toxic effects were encountered by Herrell and Nichols [16] after short term use of strepto-

13. Mushett, C. W., and Martland, H. S.: Federation Proc. **5**:194, 1946.

14. Hinshaw, H. C., and Feldman, W. H.: Proc. Staff Meet., Mayo Clin. **20**:313, 1945.

15. Lawrence, E. A.: Personal communication to the authors.

16. Herrell, W. E., and Nichols, D. R.: Proc. Staff Meet., Mayo Clin. **20**: 449, 1945.

mycin in 45 patients. In limited studies of affected dogs no lesions have been observed in the eighth nerve or in the brain. Studies on the vestibular and auditory end organs may reveal pertinent information on this matter.

The degree of local tissue damage following the parenteral administration of streptomycin concentrates showed a direct relation to the potency (purity) of the material injected. Whereas a low potency sample produced marked damage, samples of higher potency were without injurious effect. The more purified samples of streptomycin are virtually free of the toxic component which, in earlier samples of lesser purity, was responsible for a histamine-like action.[17]

Since the local damage and histamine-like action of certain streptomycin concentrates can be ascribed to impurities, there remains the possibility that the hepatoxic, renotoxic and neurotoxic effects seen in animals treated with streptomycin may also be due to impurities. Further studies with chemically pure streptomycin will serve to elucidate this possibility.

SUMMARY

The parenteral administration of highly purified as well as average streptomycin samples resulted in fatty metamorphosis of the liver in monkeys and dogs. Large doses of streptomycin concentrates produced small foci of necrosis in the livers of a few dogs.

Fatty metamorphosis was observed less often in the kidneys of monkeys and dogs treated with streptomycin. Albuminous detritus appeared in the subcapsular spaces and the tubules of the kidney in some of the monkeys. Hyaline and granular casts, epithelial cells and occasionally blood cells were seen in the sediment of the centrifuged urine of dogs receiving large doses of streptomycin concentrates. Focal tubular necrosis occurred in the kidneys of one dog.

The fatty change observed in the liver and the kidney was found to be reversible. It was not followed by permanent pathologic damage.

Complete studies of the blood of monkeys, dogs and rats treated by injection of streptomycin revealed as the only significant change slight normocytic anemia, which disappeared on cessation of dosage.

Prolonged administration of streptomycin concentrates of average purity (230 to 310 micrograms of streptomycin base per milligram of solids) resulted in neurotoxic effects in dogs, manifested by disturbances of equilibrium and possibly of auditory acuity. Spontaneous recovery occurred in 1 animal. No lesions which could explain these effects were evident in the limited material studied. Streptomycin samples of higher potency (640 to 710 micrograms of streptomycin base per milligram of solids) did not produce neurotoxic symptoms in dogs.

17. Molitor and others.[3b] Herrell and Nichols.[16]

Case Reports

PRIMARY PULMONARY VASCULAR SCLEROSIS

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THE CONDITION to be described is one which seems to us to fit into primary pulmonary vascular sclerosis as defined by Brenner.[1] The requirements listed by Brill and Krygier [2] in their recent review of the subject are (1) significant hypertrophy of the right ventricle but not of the left and (2) absence of all factors commonly believed to cause secondary vascular sclerosis or pulmonary hypertension, such as mitral stenosis or chronic pulmonary disease.

REPORT OF A CASE

A married woman 56 years of age entered the Robert Breck Brigham Hospital, Nov. 24, 1942, because of dyspnea and pain in the lower part of the chest and the back. Since her climacterium, at the age of 37, she had become increasingly irritable emotionally. She also had noted increasingly severe symptoms of what was diagnosed as Raynaud's disease by a number of physicians whom she consulted. During the summer and fall prior to her admission she had become breathless and on exertion had noted pain across the lower part of the chest and the back. She had been constantly tired and had had a nonproductive cough.

Her mother died at the age of 64 from a "shock" as did her father at the age of 54. The patient thought that her mother had had symptoms similar to her own.

The patient was a well developed, moderately obese white woman (height, 162.5 cm.; weight, 65.9 Kg. at autopsy). She was dyspneic and cyanotic. Her hands and feet showed marked cyanosis. Slight stimuli, such as touching the feet or the hands with a cold object, produced pallor. The skin of the backs of the hands, the forearms and the soft parts of the hands and fingers seemed to be coarse and thickened. The skin of the feet and toes was similar. There was no clubbing of the fingers or the toes. There was moderate edema of the lower extremities. The heart seemed to be enlarged to the right, but there were no murmurs. Expansion of the lungs was good. There were some rales at both bases. The liver was palpable.

The patient's red blood cell count was 5,170,000. Her hemoglobin concentration was 14.2 Gm. per hundred cubic centimeters of blood. The white blood cell count, the urine and the results of the routine blood serum studies were not remarkable. Roentgenograms taken on November 27 showed an enlarged heart with fulness over the pulmonary conus. Electrocardiograms showed marked right ventricular preponderance.

From the Mallory Institute of Pathology, Boston City Hospital, and the Robert Breck Brigham Hospital.

1. Brenner, O.: Arch. Int. Med. **56**:211, 457, 724, 976 and 1189, 1935. (See section on primary pulmonary vascular sclerosis, pp. 976-990.)

2. Brill, I. C., and Krygier, J. J.: Arch. Int. Med. **68**:560, 1941.

The patient improved for a while on rest in bed. A week prior to death her left leg became swollen and tender. She was thought to have acute phlebitis. The patient continued to be more and more dyspneic and cyanotic until her death on December 24.

Autopsy (thirteen hours post mortem).—The body was that of a well developed, moderately obese white woman. There was moderate pitting edema of the feet, the legs and the thighs, more marked on the left. The tips of the fingers were somewhat blue, and the overlying skin appeared to be atrophic. There was no clubbing of the fingers.

There was 500 cc. of clear yellow fluid in the right pleural cavity; the left was dry.

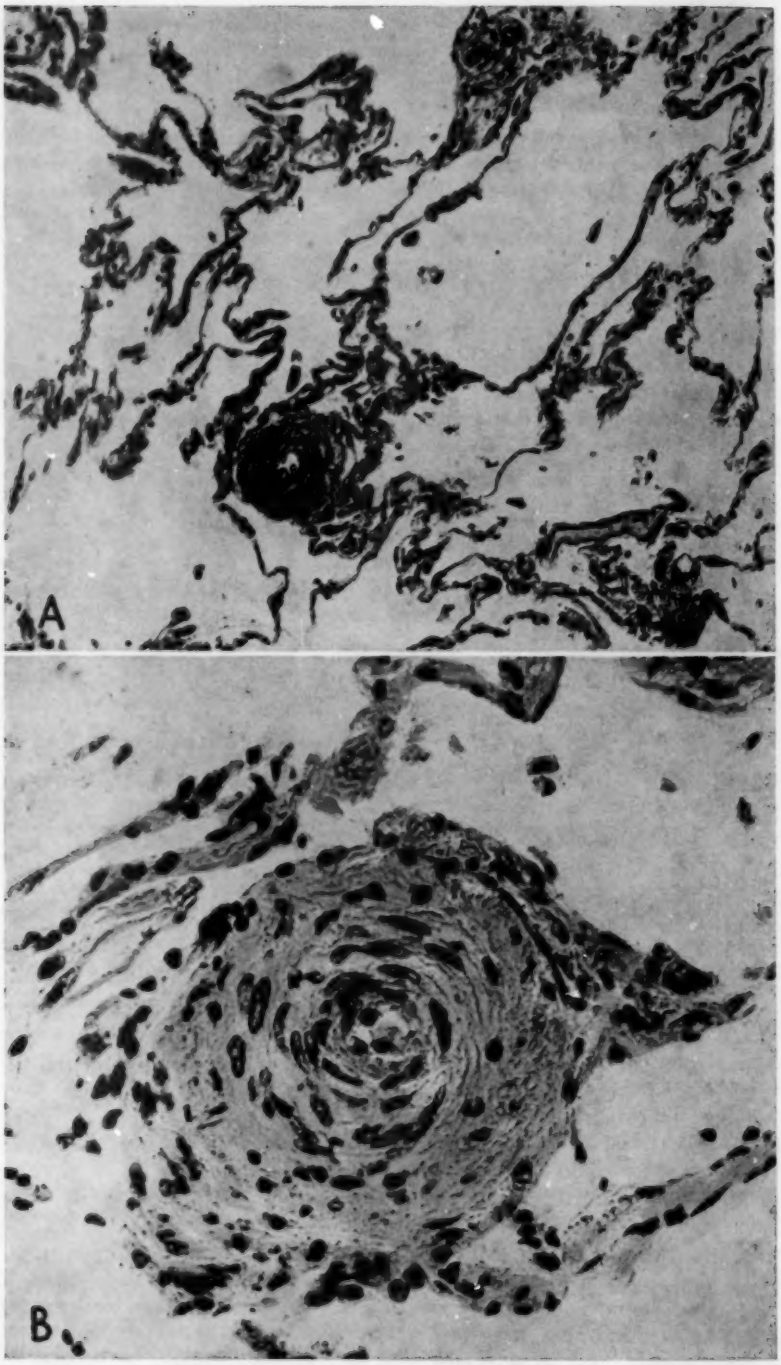
The heart weighed 280 Gm. It was not remarkable except for marked hypertrophy of the right ventricle, which measured 0.5 to 0.7 cm. in thickness. The right ventricle and auricle were somewhat dilated, and the right auricle was moderately hypertrophied. The right auricular appendage was filled with an antemortem thrombus which showed piriform softening.

The right lung weighed 430 Gm.; the left, 270 Gm. The branch of the pulmonary artery to the right lung was completely filled with thrombi of varying ages. The most recent was attached to the arterial wall and not readily torn free. The lungs were dry and crepitant throughout except for a wedge-shaped area of consolidation which extended from the hilus of the right lung to involve most of the diaphragmatic surface of the lower lobe.

The spleen weighed 120 Gm. and was acutely congested. The gastrointestinal tract was not abnormal except for a small rectal polyp. The liver weighed 1,220 Gm. The cut surface oozed considerable dark blood and revealed dark red central lobular zones, which were slightly depressed. The kidneys, the adrenal glands and the genital organs were not remarkable except for congestion. The left iliac vein was found obstructed with an antemortem thrombus, which was not firmly attached to the vessel wall.

Microscopic Examination.—The myocardium, the epicardium and the endocardium were not abnormal. The right auricle contained an antemortem thrombus, which showed early peripheral organization. The spleen, the liver and the kidneys were markedly congested. There was some central hemorrhagic necrosis of liver cells. The skin of the right forefinger was within normal limits.

The pathologic changes of interest were in the lungs. Sections of the lower lobe of the right lung revealed an area in which the alveoli were filled with red cells and in which there was infarct necrosis of the alveolar walls. The histologic picture in multiple sections taken from the remainder of the right lung and from the left lung was essentially the same. There was moderate emphysema, as shown by dilated, thin-walled alveoli. There was no thickening of the interalveolar septums. The main pathologic development in the lungs was a marked proliferation of the endothelium of the majority of the arterioles. This proliferation was of the type seen in rapidly progressive nephrosclerosis. The endothelial cells were arranged in concentric rings, which resulted in the narrowing of the lumens of the arterioles, occasionally to the point of apparent obliteration. However, no areas of necrosis were noted in the arteriolar walls. Sections through the larger branches of the pulmonary arteries showed moderate atheromatous changes in the intima. There was no evidence of left-sided heart failure in any of the sections of lung that were studied. All alveoli were well aerated and contained no edema fluid or "heart failure" cells. The vessels in the alveolar walls were not congested.



A shows the moderate alveolar dilatation observed in the lung and three small vessels with proliferative changes. Phloxine and methylene blue; × 100.

B shows the detail of the proliferative changes in an arteriole of the lung. Phloxine and methylene blue; × 450.

Elastic tissue stains revealed no alteration in the elastic fibers. Stains with the Lee-Brown modification of Mallory's aniline blue collagen stain did not reveal any unusual changes in the alveolar walls.

Permission to report this case was given us by Dr. Burton F. Hamilton, who also supplied the summary of the clinical record.

COMMENT

Clinically it was thought that the patient had a peripheral vascular disease (Raynaud's disease) which had also affected the pulmonary circulation with resultant dilatation of the heart (cor pulmonale) and failure of the right side of the heart. Five cases of this sort have been reported recently,[3] in one of which an autopsy was performed. The microscopic alteration of the lungs in the last-mentioned case consisted of marked fibrosis of the alveolar walls with minimal involvement of the vessels; the heart was moderately enlarged but did not show cor pulmonale.

In the present case the observations were not at all similar. There was no evidence of pathologic change in the digital skin examined, and the findings of interest were confined to the heart and the pulmonic vascular tree rather than to the alveolar walls. There was moderate atherosclerosis of the larger arteries with marked proliferative arteriolosclerosis and moderate right ventricular hypertrophy. There were no vascular changes similar to those seen in the lung. Although there was moderate emphysema in the other organs, we believe that the pulmonary hypertensive vascular disease in this case cannot be explained on that basis.

As is the case in peripheral arterial hypertension and arteriosclerosis—more especially that of the rapidly progressive type—pulmonary arteriolar and arterial hypertension undoubtedly precedes arteriolar and arterial changes, which may therefore be considered resultant rather than causative. The cause of pulmonary hypertensive vascular disease is unknown at present as is the cause of so-called essential hypertension. As Brenner[1] stated:

> . . . it seems unlikely that the hypertrophy of the right ventricle and the heart failure are directly due to the lesions in the pulmonary vessels, since similar symptoms and hypertrophy of the right heart may occur without pulmonary vascular lesions, and lesions greater than those in many of the cases that have been reported may occur without hypertrophy of the right ventricle or symptoms of heart failure. . . . It is possible that the pulmonary vascular lesions and the ventricular hypertrophy and failure are due to some unknown common cause rather than that they are related as cause and effect.

3. Linenthal, H., and Talkov, R.: New England J. Med. **224**:682, 1941; **227**:433, 1942.

Pathologically, primary pulmonary vascular sclerosis is not as clear-cut a picture as might be expected from the rather dogmatic criteria cited in the introduction. As emphasized by Brenner, the lesions are varied. Lesions of the intima of the larger arteries predominate in some cases; lesions of the endothelial lining of the smaller arteries and arterioles, in others. In some cases the lesions are localized in one portion of the lung. In others the lesion seems to be one primarily of the elastica and the media.

Thus, as suggested by Brill and Krygier, this problem is not at present susceptible to explanation by histologic methods. Perhaps, as they also suggested, when a means of determining the tension in the pulmonary circuit is developed, one may be many steps closer to the solution of this problem.

SUMMARY

In the case of primary pulmonary vascular sclerosis described, the most important pathologic change was proliferative pulmonary arteriolosclerosis, which probably resulted from hypertensive pulmonary vascular disease.

PRIMARY ADENOCARCINOMA OF THE UMBILICUS

GERARD DESFORGES, M.D., BOSTON

ACCORDING to Cullen,[1] cancer of the umbilicus, whether primary or secondary, is exceptionally rare. This fact is borne out by the finding of only 9 such tumors in 18,668 autopsies and 123,825 surgical specimens seen at the Mallory Institute of Pathology of the Boston City Hospital.

Tumors of the umbilicus are classified by Cullen [1] as follows:

A. Primary umbilical carcinoma
   1. Squamous cell carcinoma
   2. Adenocarcinoma

B. Secondary umbilical carcinoma
   1. From the stomach
   2. From the gallbladder
   3. From the intestine
   4. From the ovaries
   5. From the uterus
   6. From other abdominal organs

Perhaps owing to the rarity of its occurrence, the possibility of adenocarcinoma primary in the umbilicus is often overlooked, particularly if signs or symptoms suggestive of visceral tumor are present, leading to the conclusion that the umbilical lesion is secondary.

The case to be reported is an instance of a primary umbilical lesion in which, in the presence of intestinal symptoms, a biopsy report of adenocarcinoma led to the erroneous belief that metastatic disease was present. Hence, surgical removal was not attempted.

A short review of the embryology and the anatomy of the umbilical region will not only show the possible origin of adenomatous lesions but indicate why a primary tumor of the umbilicus is so rare.

As the embryo enlarges, its ventral unclosed area becomes relatively smaller. This region at the junction of the embryonic and the extra-embryonic territory is the primitive umbilicus.[2] Passing through this primitive structure are the omphalomesenteric or vitelline duct, the allantois and the accompanying blood supply. The allantois is a true vestigial structure and quickly disappears only to leave a few fibrous strands connecting with the urachus at the umbilicus. Anomalous development of these structures leads to such well known umbilical

From the Mallory Institute of Pathology, Boston City Hospital.

1. Cullen, T. S.: The Umbilicus and Its Diseases, Philadelphia, W. B. Saunders Company, 1916, pp. 400-448.

2. Arey, L. B.: Developmental Anatomy, Philadelphia, W. B. Saunders Company, 1942, p. 108.

defects as fecal fistula (persistent patency of the omphalomesenteric duct) and to urinary fistula (persistently patent urachus).

Histologically, glandular epithelium may be observed in both patent urachi and persistent omphalomesenteric ducts.[3] This observation may well be expected when it is realized that the omphalomesenteric duct is directly connected with the primitive midgut and that the early hindgut possesses the anlage of both the rectum and the urachus. As for the urachal remnant, its epithelium does not become specialized but retains the primitive cell's potentiality of differentiating into any epithelial cell type.[4]

Normally these fetal structures proceed to total obliteration, and the umbilicus becomes a small circular pad of dense connective tissue covered by thin squamous epithelium. It is devoid of hair follicles, sweat glands and sebaceous glands. Therefore, save for the rare remnants of omphalomesenteric duct and urachus which may persist, no structures exist from which adenocarcinoma may arise.

REPORT OF A CASE

A 79 year old single white woman entered the Boston City Hospital, April 11, 1945, with the chief complaints of weakness, loss of weight, swelling of the abdomen and some abdominal pain present during the last four months.

About one year before entry she noticed discoloration of the umbilicus, with pruritus. This subsided after two months. During the whole year, however, she had increasing constipation and pain in the back which radiated down the legs and was worse at night. Five weeks before entry discoloration and pruritus of the umbilicus reappeared, and now there was also a malodorous yellowish discharge. Two weeks before entry the discharge subsided, but the swelling remained. Epigastric distress, gaseous eructations, crampy pain, fulness and regurgitation of food were prominent during the few weeks before entry.

A physician made the diagnosis of abdominal neoplasm with metastasis to the umbilicus and sent the patient to the hospital.

The familial social and past histories, as well as the system review, were noncontributory.

The examination on this, the first, admission showed a small elderly woman appearing chronically ill. Her temperature was 98.6 F.; pulse rate, 80; respiration, 20; blood pressure, 200 mm. of mercury systolic and 80 mm. diastolic. The skin was dry, warm and smooth, with loss of subcutaneous tissue. There was limitation of motion of spine and hips. The fundi showed arteriosclerotic and hypertensive changes. The heart was enlarged and showed grade I apical and basal systolic murmurs. The abdomen was rounded and distended, with hyperactive peristalsis. In the midabdominal region there was minimal tenderness. Palpation further revealed a poorly defined, firm, relatively fixed mass which was presumably attached to the firm, rounded, granulating, 1.0 by 2.0 cm. lesion at the umbilicus. Pelvic and rectal examinations disclosed no abnormality.

The hemoglobin content was 80 per cent; the white blood cells numbered 7,000 per cubic millimeter, with polymorphonuclear leukocytes 60 per cent; the nonprotein nitrogen of the blood was 29 mg. per hundred cubic centimeters; the total protein, 5.8 Gm. per hundred cubic centimeters; the Hinton test was negative; two

3. Trimingham, H. L., and McDonald, J. R.: Surg., Gynec. & Obst. **80**: 152, 1945.

4. Rappoport, A. E., and Nixon, C. E.: Arch. Path. **41**:388, 1946.

stool specimens submitted to the guaiac test gave a negative result; a guaiac test of vomitus gave a 2 plus reaction; urine specimens concentrated to 1.010 and showed only an occasional white blood cell. A roentgenographic series made to reveal metastatic growths showed only arthritic changes. A gastrointestinal series was made with use of a barium sulfate enema but reported as unsatisfactory. A biopsy specimen of the umbilical lesion taken at this time showed adenocarcinoma.

The course in the hospital with symptomatic and supportive therapy was relatively uneventful. It was felt that adenocarcinoma appearing at the umbilicus meant that wherever the primary lesion was it was inoperable, and therefore the patient was discharged to a nursing home on the thirtieth hospital day. The surgeons, in consultation, felt that operation was inadvisable.

Approximately six months later, October 23, the patient reentered the Boston City Hospital because of inability to eat solid food. Abdominal pain had become severe, and she vomited frequently, shortly after meals. To alleviate pain, morphine sulfate was being given twice daily at the time of this admission.

Examination at this time showed only more emaciation and slight disorientation. The temperature was 98.0 F.; the pulse rate, 110; the respiratory rate, 12; the blood pressure, 135 systolic and 55 diastolic. The white blood cell count was 6,400, with 85 per cent polymorphonuclear leukocytes, 12 per cent lymphocytes and 3 per cent monocytes. The abdomen was boardlike. The umbilical lesion was fungating and had increased to 2.0 by 2.0 cm. in size. It bled easily when rubbed with a throat stick.

Since the lesion was still felt to be secondary, the patient was again given symptomatic and supportive therapy. She gradually lapsed into a stuporous condition and quietly died on the third day after her second admission, approximately a year and a half after the initial development of symptoms.

The clinical diagnoses were: adenocarcinoma of the umbilicus, primary site undetermined; ? terminal peritonitis; general arteriosclerosis; hypertrophic arthritis; arteriosclerotic heart disease with cardiac enlargement, normal sinus rhythm, compensated, class 2; complete absence of teeth.

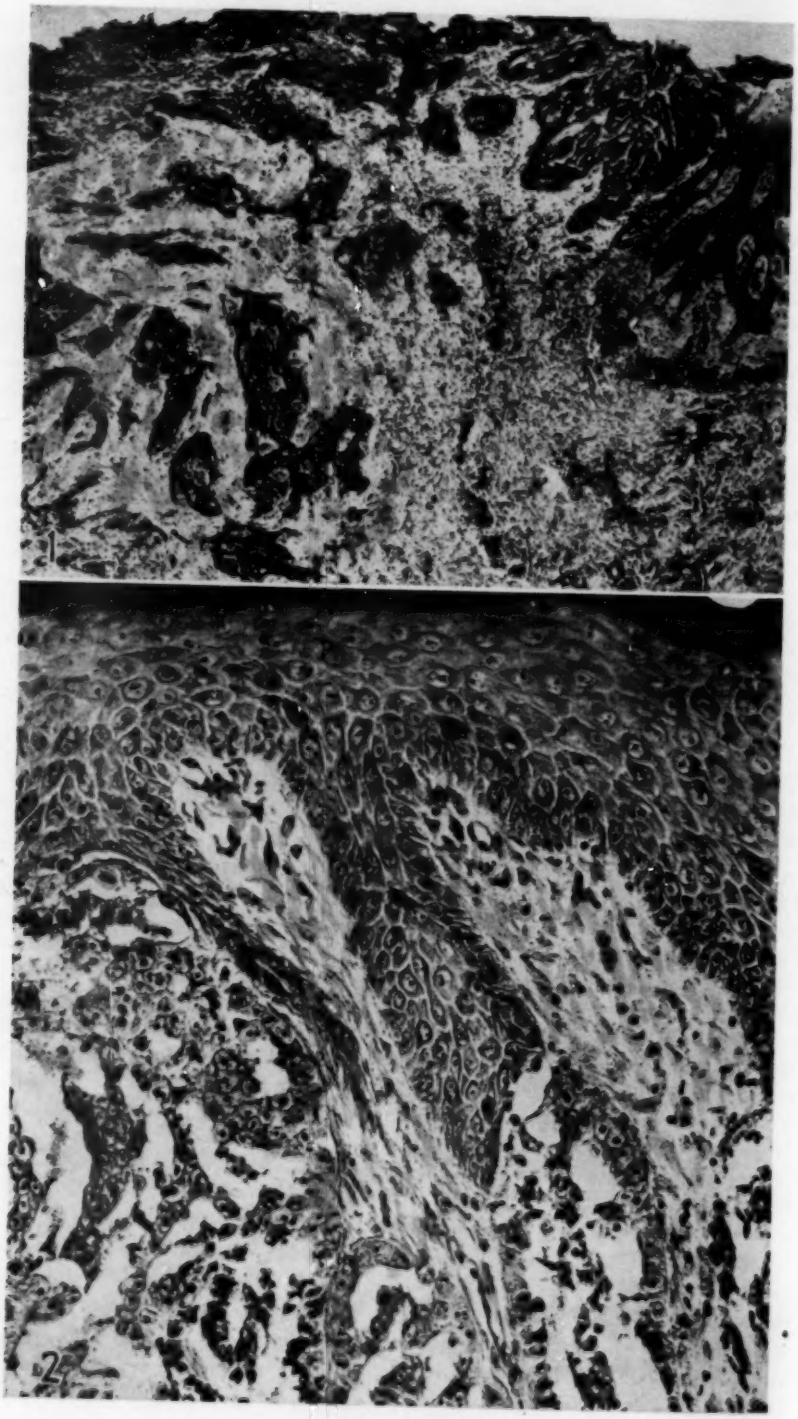
Autopsy.—Only the pertinent gross and microscopic postmortem observations will be presented. The body was that of a fairly well developed, cachectic female weighing 90 pounds (41 Kg.). In the umbilical area there was a blackened, crusted 2.0 cm. area which exuded sanguinous material on pressure. No fistulous tract was demonstrable. There were no other external masses.

The peritoneal cavity was filled with thick, viscid yellowish green material collected into pockets, which were successively broken with manipulation. All intestinal loops were interadherent and also plastered against the abdominal walls by intervention of a gray-green and dark red fibrinopurulent material. The domes of the diaphragm were rendered adherent to the liver on the right and to the spleen on the left by the same material.

The gastrointestinal tract revealed only a 2.5 cm. ulcer with a smooth base and rolled edges in the first portion of the duodenum.

No other visceral masses or tumors were found.

Microscopic Examinations.—The biopsy specimen taken approximately six months before death showed a slowly growing, well differentiated adenocarcinoma made up of columnar and cuboidal cells, invading the subcutaneous tissue and associated with a marked overlying chronic inflammatory reaction. The tumor was forming glands and tubular spaces, which were separated from each other by fine strands of connective tissue. The tumor cells were large, with slight variation



(See legends on opposite page)

in size. The cell outlines were not clearly discernible. The cytoplasm was palely basophilic. A large vesicular nucleus occupied almost the entire cell volume. The nucleolus was dark and dense. Mitoses were rare. No remnants of omphalomesenteric duct or of urachus were discernible.

The section of tumor taken at autopsy six months later was similar to the biopsy specimen, but now there was evidence of more rapid growth. The gland spaces were not so well formed; there was more irregularity of cell size, with occasional multinucleate giant cells and more frequent mitoses. The associated inflammatory reaction was more intense.

Examination of the edge of the duodenal ulcer revealed only acute and chronic inflammatory reaction with no evidence of cancer.

In the subdiaphragmatic collagen were noted several lymphatic spaces which were either lined with or contained large pale vesicular cells with very large nuclei and dense nucleoli, similar to those seen in the original biopsy specimen. Mitoses were rare here also.

Final Anatomic Diagnoses.—Adenocarcinoma of the umbilicus; metastasizing to subdiaphragmatic lymphatics; fibrinopurulent peritonitis, with old and fresh subdiaphragmatic abscesses and healed and acute perisplenitis; chronic duodenal ulcer; brown atrophy of the myocardium; benign nephrosclerosis; cystic left ovary.

Bacteriologic Examination.—Culture of the peritoneal fluid showed Bacillus coli, Streptococcus with alpha hemolysis and Clostridium welchii.

COMMENT

Normally, as has been mentioned, the umbilicus is such a fundamentally simple structure that carcinoma has practically no starting place except in the squamous epithelial coat. However, remnants of the omphalomesenteric duct, whose most common anomaly every one is familiar with as Meckel's diverticulum, may be found occasionally as rests in the umbilical structures, as well as urachal rests. It is undoubtedly from these vestigial remnants that primary adenocarcinoma of the umbilicus originates. Although no vestigial remnants were found in the specimens obtained at biopsy and autopsy, it is possible that they were destroyed by the tumor and the associated inflammatory reaction.

In the case presented, death was due to extensive fibrinopurulent peritonitis, which undoubtedly resulted from previous fistulization of necrotic tumor, allowing free communication between the peritoneal cavity and the outside.

The only site of tumor extension was in the subdiaphragmatic lymphatics, where the tumor resembled the slowly growing adenocarcinoma of the original biopsy. The presence of tumor cells here,

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Explanation of Figures

Fig. 1.—Low power detail of a section of an autopsy specimen, showing a margin of skin ulcerated by tumor and diffuse subcutaneous infiltration with nests of poorly differentiated adenocarcinoma. The associated inflammatory reaction is marked. × 60.

Fig. 2.—Higher power detail of a section of the biopsy specimen, showing the slowly growing, well differentiated adenocarcinoma invading the connective tissue of the umbilicus up to the germinal layer of the epithelium. × 360.

according to Wilcox and Greenblatt,[5] is one of the expected findings in a cancer of this type which has progressed to dissemination. Other possible sites of metastases are the axillary, the inguinal and even the hilar lymph nodes, which are reached via the transdiaphragmatic lymphatics.

SUMMARY

There are embryologic and histologic bases for the development of primary adenocarcinoma of the umbilicus.

In the case of primary adenocarcinoma of the umbilicus presented, death was due to peritonitis, which probably resulted from fistulization of the necrotic tumor, allowing communication between the peritoneal cavity and the outside.

Early recognition of primary adenocarcinoma of the umbilicus is important because when correctly diagnosed the lesion represents a readily curable disease.

5. Wilcox, E. A., and Greenblatt, R. B.: Am. J. Surg. **34**:116, 1936.

LIPOMA OF THE MAMMARY GLAND

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ADIPOSE tissue is a normal component of the mammary gland, yet a neoplasm consisting of adipose tissue alone, a lipoma, occurring in the mammary gland proper is exceedingly rare.[1] This impression is amply confirmed by the available reports. Menville,[2] who collected the literature on lipoma of the mammary gland, listed 24 cases from the Johns Hopkins Hospital, to which Geschickter[3] added 6. According to Geschickter, "practically all of the lipomas were superficial, that is, anterior to the mammary gland proper." Adair, Pack and Farrior[4] encountered 15 patients with lipoma of the mammary gland at the Memorial Hospital for the Treatment of Cancer and Allied Diseases and stated that the tumor was "usually retromammary." Among the patients of the Skin and Cancer Unit of the New York Post-Graduate Medical School and Hospital, de Cholnoky[5] encountered 27 with lipoma of the mammary gland. He did not state, however, the exact site of the growth. The available information thus fails to reveal the number of cases in which lipoma actually arose within the substance of the mammary gland. This paper records a case of lipoma of the mammary gland.

REPORT OF A CASE

A 34 year old white woman was admitted to the University Hospitals, Oklahoma City, Jan. 19, 1943, complaining of a mass which had been present in the left mammary gland for eight years. It had gradually increased in size. She had had some pain in the left mammary gland, associated with menses. For four months prior to admission she had suffered from a dull aching in the left side of the chest and left shoulder, relieved by wearing a brassiere. She was the mother of five children, four of whom were born after the mass was first noticed.

At the time of admission the patient was obese and appeared to be in good health. The only positive finding was a mass, about 10 by 8 cm., within the lower outer quadrant of the left mammary gland. The mass was circumscribed, freely movable, fairly firm and lobulated. It was not fixed to the skin or deeper structures. There was no retraction of the nipple, dimpling of the skin, tenderness, redness or increased local heat. The axillary lymph nodes were not palpable.

From the Department of Pathology, University of Oklahoma School of Medicine.

1. Spalding, J. E.: Guy's Hosp. Rep. **94**:80, 1945.

2. Menville, J. G.: Am. J. Cancer **24**:797, 1935.

3. Geschickter, C. F.: Diseases of the Breast, ed. 2, Philadelphia, J. B. Lippincott Company, 1945, p. 356.

4. Adair, F. E.; Pack, G. T., and Farrior, J. H.: Am. J. Cancer **16**:1104, 1932.

5. de Cholnoky, T.: Arch. Surg. **38**:79, 1939.

The mass was thought to be a fibroadenoma. On January 22, a large lobulated and encapsulated "fatty tumor" replacing the lower half of the left mammary gland was excised (by Dr. John W. Cavanaugh). The postoperative course was uneventful, and the patient was discharged on January 28. Three and one-half years later there had been no recurrence of the growth.

The specimen consisted of a lobulated, encapsulated mass of adipose tissue, 14 by 9 by 8 cm., weighing 360 Gm. (figure). The cut surfaces were composed of lobules of adipose tissue with delicate septums. Microscopic preparations stained with hematoxylin and eosin, representing many parts of the growth, disclosed

Lipoma of the mammary gland removed from a 34 year old white woman. The growth is composed entirely of various-sized lobules of adipose tissue.

various-sized lobules of adipose tissue cells with scant delicate septums containing blood vessels. Nowhere were any acini or ducts of the mammary gland seen. A delicate connective tissue capsule delimited the growth.

COMMENT

A focal or a diffuse increase of the adipose tissue surrounding the acini and ducts of one or both mammary glands is properly designated as lipomatosis. Such adipose tissue infiltration of the mammary gland is analogous to similar infiltration of the pancreas or the myocardium.

A growth immediately beneath the skin, near or overlying the mammary gland, should be classed as subcutaneous lipoma. True lipoma of the mammary gland is a focal aggregation of neoplastic adipose tissue cells delimited by a capsule and located within the mammary gland proper.

SUMMARY

A large lipoma of the left mammary gland was observed in a 34 year old white woman. A survey of the literature disclosed that true intramammary lipoma is exceedingly rare.

ACUTE BRONCHOPNEUMONIA DUE TO ASPERGILLUS FUMIGATUS FRESENIUS

Report of a Case, with a Description of Acute and Granulomatous Lesions Produced by the Fungus in Rabbits

NORMAN S. COOPER, M.D., NEW YORK

THE LITERATURE contains fairly numerous references to pulmonary aspergillosis. In the great majority of the reported cases the causative organism was Aspergillus fumigatus. In those instances in which the diagnosis was made during life the disease usually manifested itself in a syndrome clinically and roentgenologically indistinguishable from that of chronic pulmonary tuberculosis. Aspergillosis was identified when the causative organism was isolated from the sputum in the absence of the tubercle bacillus. Anatomically, in the reported cases, chronic pulmonary aspergillosis was characterized by the formation of granulomas (often with giant cells), by fibrosis and by cavitation.[1]

In the older literature a few cases of a more acute type of pulmonary aspergillosis were reported.[2] These cases were usually diagnosed only at autopsy. The lesions consisted of colonies of the mycelia and spores of Aspergillus surrounded by an area of necrosis, with or without a delimiting zone of polymorphonuclear neutrophils. A search of the recent literature has failed to reveal any anatomic descriptions of acute pulmonary aspergillosis.

Acute aspergillar bronchopneumonia was an unexpected finding in a recent autopsy at this hospital.

REPORT OF A CASE

A 45 year old, white, Italian-born barber was admitted to the New York Hospital complaining of severe epigastric pain. A chronic duodenal ulcer had been diagnosed by fluoroscopic and roentgen examinations on previous admissions. Laparotomy was performed on the day of admission, and a ruptured duodenal ulcer was plicated.

The patient's temperature rose to 40.3 C. (104.5 F.) on the third postoperative day, and thereafter it ranged between 37.7 and 40.4 C. (99.8 and 104.7 F.) despite the administration of sulfadiazine (a blood level of 6.8 mg. per hundred cubic centimeters was attained). On the sixth hospital day laparotomy was done a second time; pus was seen oozing from the site of the previous plication. On the fourteenth hospital day jejunostomy was performed to permit feeding by tube.

From the New York Hospital and the Department of Pathology, Cornell University Medical College.

1. (*a*) Renon, L.: Étude sur l'aspergillose chez les animaux et chez l'homme, Paris, Masson & Cie, 1897. (*b*) Wätjen, J., in Henke, F., and Lubarsch, O.: Handbuch der speziellen pathologischen Anatomie und Histologie, Berlin, Julius Springer, 1931, vol. 3, pt. 3, p. 481.

2. Saxer, F.: Pneumomykosis Aspergillina, Jena, Gustav Fischer, 1900. Wätjen.[1b]

Subsequently the patient became weaker and more lethargic. The original operative wound, which had never healed, drained bile-containing fluid.

Since the seventh hospital day the patient's respirations had ranged between 32 and 40 per minute, and there was occasional cyanosis. On the fifteenth hospital day a nonproductive cough, rhonchi and rales developed and persisted. The patient became cyanotic and stuporous on his twentieth hospital day and died on the twenty-first day.

Roentgenograms of the chest made on the second, fourth and sixth hospital days were interpreted as showing atelectasis and pneumonic consolidation at the base of the right lung.

Autopsy.—There was generalized peritonitis. Immediately beyond the pylorus a gaping circular perforation, 12 mm. in diameter, was seen in the anterior wall of the duodenum. There were silk sutures in the indurated margins of the perforation.

There were fibrinous adhesions between the right lung and the diaphragmatic pleura. The lungs together weighed 1,530 Gm. On cut surfaces the upper and lower lobes of both lungs were studded with firm, white, raised nodules, 1 to 2 mm. in diameter. From many of the nodules pus exuded on pressure. The process was most marked in the upper lobe of the right lung. The middle lobe of this lung was atelectatic, though practically free from the nodular lesions.

Microscopic Observations.—The lungs contained many small abscesses in bronchi and immediately adjacent alveoli (fig. 1). Alveoli around the abscesses contained neutrophils, mononuclear phagocytes and small amounts of fibrin. In the centers of the abscesses were what appeared to be sporulating hyphae. These were faintly basophilic, translucent and about 7 to 10 microns in diameter and 30 to 120 microns long. Many of them had joints resembling those of a bamboo pole, and some branched one or more times (fig. 3). A few basophilic round bodies were free in the abscesses. The hyphae had the same appearance in hematoxylin-eosin, Brown,[2a] Mac-Callum [2b] and Giemsa stains. No bacteria were seen.

Culture of the peritoneal exudate yielded hemolytic Staphylococcus aureus. Cultures of material from the upper lobe of the right lung, made originally in liver-dextrose broth and transferred later to Sabouraud's agar and blood agar, showed Aspergillus fumigatus Fresenius in pure growth except for one colony of Staphylococcus albus. Dr. Kenneth B. Raper, of the Agricultural Research Administration, United States Department of Agriculture, identified the fungus.

EXPERIMENTS

Inoculations showed that this strain of A. fumigatus is pathogenic for rabbits. The fungus was transplanted to dextrose agar plates, and the subcultures were grown at 30 C. for five to seven days. A heavy suspension of spores of the organism was prepared by washing off the surfaces of agar plates with a small quantity of Locke's solution. The rabbits were anesthetized with ether, and 1 cc. of the spore suspension was injected intratracheally. The animals were maintained under anesthesia with their heads elevated for an hour after the operation. All of them appeared to be normal postoperatively. They were killed at varying periods.

Twenty-four and forty-eight hours after the intratracheal injections sections of the lungs showed a histologic picture (fig. 2) identical with that encountered

2a. Brown, J. H., and Breun, L.: Bull. Johns Hopkins Hosp. 48:69, 1931.

2b. Mallory, F. B.: Pathological Technique, Philadelphia, W. B. Saunders Company, 1938, p. 274.

in the autopsy described in the foregoing pages except that only a few colonies of the fungus were seen. In these the hyphae were paler staining, showed a lesser tendency to branch and were smaller than those in the reported case.

In animals killed six to twenty-six days after inoculation the acute bronchopneumonic character of the lesions had disappeared. There was a granulomatous

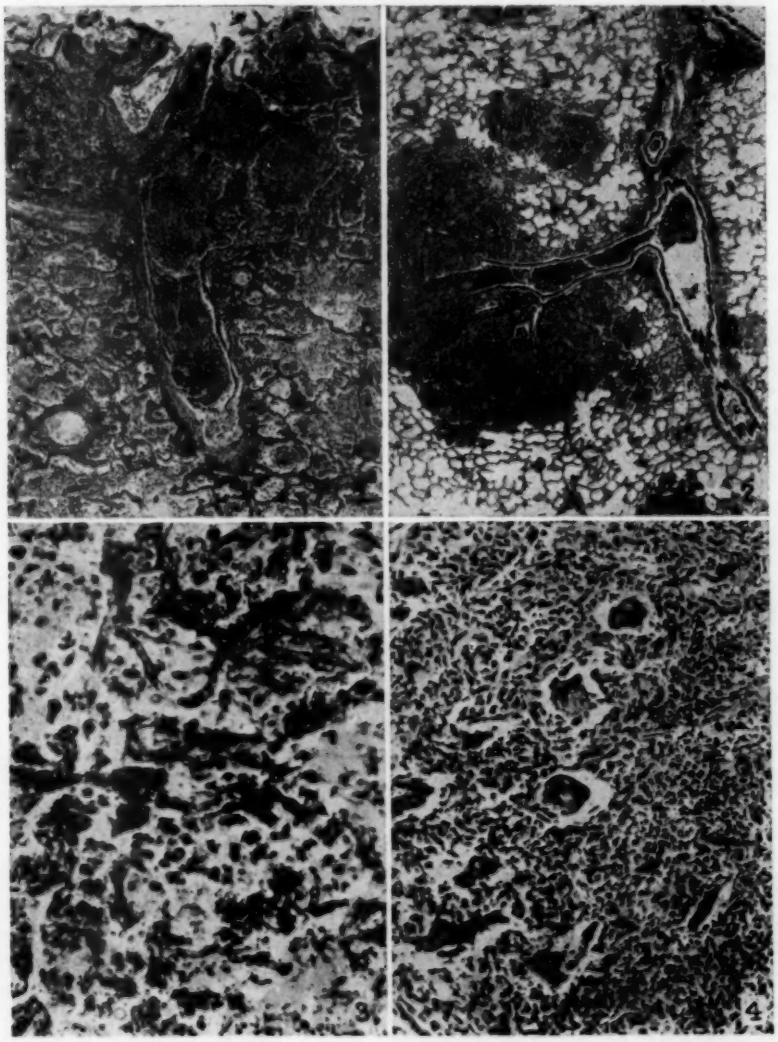

Fig. 1.—Low power view of a section from the upper lobe of the right lung of the patient.

Fig. 2.—Low power view of a section of the lung of a rabbit twenty-four hours after intratracheal inoculation of A. fumigatus.

Fig. 3.—High power view of a section from the lung of the patient, showing the hyphae of A. fumigatus.

Fig. 4.—High power view of the granulomatous reaction in the lung of a rabbit six days after intratracheal inoculation of A. fumigatus.

reaction, with masses of epithelioid cells, lymphocytes and giant cells (fig. 4). There were large necrotic areas, bordered by collagen. The lesions were slightest in the animal which had been allowed to survive longest. No lesions at all were found in the lungs of a rabbit which was killed seventy-three days after inoculation. No organisms were seen in the sections from this group of animals, but A. fumigatus in pure growth was obtained in cultures of material from the lungs in every instance except that of the rabbit which had been allowed to survive for seventy-three days.

Rabbits which were given 5 cc. of the spore suspension intravenously died within twenty-four hours. Miliary abscesses were found in the heart, the lungs and the liver. Postmortem blood cultures showed A. fumigatus in pure growth, but no hyphae were seen in histologic sections.

COMMENT

For some years, a controversy existed over whether pulmonary aspergillosis was ever a primary disease. Because of the ubiquity of Aspergilli and the rarity of aspergillosis, many authors found it difficult to accept the thesis that the fungi could invade undamaged lung tissue. Others pointed out that repeated exposure to large numbers of spores might be necessary for infection. Renon [1a] observed aspergillosis in wigmakers and pigeon feeders, an interesting fact being that the latter took grain into their mouths, chewed it and then forced it into the mouths of pigeons. The wigmakers used corn meal for freeing the hair with which they worked from oil. Corn meal, grain and the mouths of pigeons are all rich sources of Aspergilli.

In many of the cases reported in the older literature there were other coexisting lesions of the lungs, such as tuberculosis, infarcts, bacterial pneumonia or pulmonary abscess. In most of those in which the aspergillosis was called primary, attempts to rule out other lesions, particularly tuberculosis, by cultural methods were inadequate. In at least one of Renon's patients whose aspergillosis was reported as primary, it was subsequently shown to be associated with tuberculosis.[3] More recently, few cases of pulmonary aspergillosis in which autopsies were made have been reported. In 1923 Lang and Grubauer published the details of a case in which aspergillosis appeared to be secondary to bronchiectasis.[4] In 1926 Macaigne and Nicaud [5] described a case of chronic aspergillosis in which careful search failed to reveal tuberculosis or other complicating lesions. Most authors have accepted this work as demonstrating that pulmonary aspergillosis can occur as a primary disease. However, the possibility remains that the traces of a preexisting disease might have been obliterated by the unusually extensive fibrosis which was present.

In the case reported here, there was no evidence of the presence of any pulmonary lesion other than aspergillosis, but the patient had had peritonitis for at least the last two weeks of life. Wätjen [1b] discussed the possibility that debility may be the predisposing factor in otherwise primary pulmonary aspergillosis.

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3. Sergent, cited by Macaigne and Nicaud.[5]

4. Lang, F. J., and Grubauer, F.: Virchows Arch. f. path. Anat. **245**:480, 1923.

5. Macaigne, M., and Nicaud, P.: Presse méd. **34**:401, 1926.

In the experiments described in this report the seeding of the organisms in the lungs of the rabbits may have been facilitated by prolonged ether anesthesia. Under these conditions the organism isolated from the patient's lungs proved capable of inducing acute bronchopneumonia in healthy rabbits. However, the disease soon became granulomatous, and it later disappeared entirely. At no time did the animals which had received intratracheal injections of the suspension of spores appear to be ill.

SUMMARY

A case is presented in which acute bronchopneumonia due to A. fumigatus was an unexpected finding in a debilitated patient. No lesions other than those due to aspergillosis were found in the lungs. The organism was seen in histologic sections and isolated in virtually pure culture from the lungs. Similar bronchopneumonia was produced in rabbits by intratracheal injections of a suspension of spores of the organism thus obtained, and the fungus was obtained in pure culture again from the rabbits' lungs. After the lapse of several days the lesions in the rabbit became granulomatous; eventually they disappeared.

Laboratory Methods and Technical Notes

SILVER IMPREGNATION OF SPIROCHETES IN TISSUE SECTIONS

Description of a New Technic

MADUREIRA PARÁ, M.D., RIO DE JANEIRO, BRAZIL

NUMEROUS modifications of the original Levaditi [1] (1905) method of demonstrating spirochetes in tissues have been proposed, all aimed at simplifying the procedure, making it more rapid or offering a wider range of application.

A modification suggested by Warthin and Starry [2] for staining paraffin-mounted tissue sections deserves special mention. The sections, fixed on cover slips, are sensitized with ferrous ammonium sulfate or with ferric alum (iron and potassium sulfate) before being treated with silver nitrate. Impregnation is brought about through selective capillary action, since the cover slip, with the section mounted on it, is covered by a second cover slip of equal size before it is placed in the silver solution. Another interesting technic, applicable either to frozen or paraffin sections, has been devised by Dieterle.[3] It consists of coating the section with gum mastic, thus giving it the physical characteristics of a true tissue block. However, the practical difficulties and the failures which often result when one is employing these two technics render them unsuitable for ordinary diagnostic purposes.

Krajian [4] described an improvement on Dieterle's method, but one applicable only to frozen sections. Steiner and Steiner [5] suggested modifications which are applicable only to paraffin sections mounted on slides. In my experience the Steiner modification represents the easiest and most reliable method so far described. However, the necessity of using sodium and potassium tartrate as well as gum mastic limits its application, since neither of these substances is commonly available in histologic laboratories. Furthermore, gum mastic is, on occasion, difficult to obtain.

THE COLLOIDAL SILVER–LITHIUM IMPREGNATION TECHNIC

After making many attempts to find a technic for impregnation which would be reliable, easily executed and time saving, I finally developed a new method which can be applied in either of two processes, one slightly more complex and consequently more time consuming than the other. Both are recommended, since the results obtained by either proc-

From the Histopathological Section of the National Yellow Fever Service.

1. Levaditi, C.: Compt. rend. Soc. de biol. **59**:326, 1905.
2. Warthin, A. S., and Starry, A. C.: Am. J. Syph. **4**:97, 1920.
3. Dieterle, R. R.: Arch. Neurol. & Psychiat. **18**:73, 1927.
4. Krajian, A. A.: Histological Technic, St. Louis, C. V. Mosby Company, 1940, p. 163.
5. Steiner, G., and Steiner, G.: J. Lab. & Clin. Med. **29**:868, 1944.

ess are in complete accord with those obtained by the original Levaditi or the Steiner method.

This new process is applied to paraffin-embedded sections mounted on slides. Preferably tissues should have been fixed in solution of formaldehyde U. S. P. diluted 1 : 10 in saline solution.

BASIC METHOD

Solutions Required.—1. Uranium nitrate: A 1 per cent solution in water. This is stable at room temperature.

2. Silver nitrate: A 1.5 per cent solution in water. This can be freshly prepared or stored in the dark at room temperature.

3. Silver nitrate–lithium carbonate solution: This is prepared as follows: Heat 100 cc. of a 0.2 per cent silver nitrate solution almost to boiling and add 2.0 cc. of a cold saturated (about 1.3 per cent) solution of lithium carbonate in water. The saturated lithium carbonate solution is preferably prepared a few days before use. Heat the mixture gradually and allow it to boil for one or two minutes. The resulting opalescent solution is passed through ordinary filter paper, and the clear filtrate obtained is stored in a refrigerator, where it will keep for a maximum period of one month.

4. Rosin (colophony): A 5 per cent solution in absolute alcohol. This solution should be preserved in a refrigerator.

5. Levaditi's reducing solution: This has the following formula:

Pyrogallic acid	4.0 Gm.
Solution of formaldehyde U.S.P.	5.0 cc.
Distilled water	100.0 cc.

Procedure.—1. Cut paraffin-embedded sections of tissue 4 to 6 microns thick and fix on slides. Remove the paraffin and hydrate in the usual manner.

2. Place the sections in the uranium nitrate solution and leave at room temperature for thirty minutes.

3. Wash rapidly in distilled water.

4. Treat with the silver nitrate solution for two hours in an incubator at 56 C., replacing the solution at the end of the first hour with fresh silver nitrate. Or, as an alternative, the sections may be treated at 37 C. overnight without changing the solution.

5. Wash rapidly in distilled water.

6. Transfer to the silver–lithium colloidal preparation and treat for one hour at room temperature. The mixture is prepared in a 50 cc. Coplin jar immediately before using, in the following manner:

Silver nitrate-lithium carbonate solution	45 cc
5 per cent alcoholic solution of rosin	5 cc

The rosin solution is added rapidly to the silver nitrate–lithium carbonate solution. The mixture is shaken gently, and a milky colloidal suspension results.

7. Transfer the sections directly to the Levaditi reducing solution and allow them to stand at room temperature for ten to fifteen minutes.

8. Wash rapidly in distilled water.

9. Examine the slide under the microscope to determine whether the tissue has been properly impregnated. If not, treat the section rapidly with absolute alcohol

and repeat step 6 for ten minutes, then follow with another reduction lasting five to ten minutes.

10. Dehydrate and mount in Canada balsam.

Sections impregnated in this manner have the yellow-brown color characteristic of sections stained by Levaditi's original method. The cell nuclei stain brown, and the spirochetes stain black.

It occasionally happens that some sections fail to become well impregnated; this is shown by an excessive prominence of connective tissue or by the formation of a fine precipitate. Generally it is due to a faulty fixing solution. In such cases new sections are cut from the paraffin block, fixed on slides, the paraffin removed and the section hydrated in the usual manner as far as 50 per cent alcohol. The slides are then placed in a 2 per cent solution of rosin in absolute alcohol for one hour at room temperature. They are then washed in absolute alcohol, rehydrated and impregnated by the regular process.

VARIATIONS IN THE METHOD

In order that this method might be made as practical as possible, other reagents have been tested which may be used as substitutes for certain chemicals employed in the basic process. When used in the manner described, each functions in a satisfactory manner. Those which were found to give results almost as satisfactory as the basic reagents are listed here.

Substitutes for Uranium Nitrate as Mordant.—1. Ferrous ammonium sulfate: A 4 per cent solution in water is used. The sections are treated for forty-five minutes at 56 C.

2. Sulfur water: This is prepared as follows: To 200 cc. of distilled water add 10 cc. of a 5 per cent solution of anhydrous sodium bisulfite and 10 cc. of normal hydrochloric acid. The solution, which has the odor of sulfur dioxide, is kept in a tightly stoppered flask. Sections are treated with this solution for ten to fifteen minutes at room temperature.

3. Oxalic acid: A 1 per cent solution in water is used. Sections are treated for twenty minutes at 56 C.

4. Copper sulfate: A 0.5 per cent solution in water is used. Sections are treated for thirty minutes at room temperature.

5. Potassium permanganate: This is used in the form of a 1:5,000 dilution in water. Sections are treated for five minutes at room temperature.

Substitutes for Silver Nitrate–Lithium Carbonate Solution in the Sensitizer.—1 Colloidal silver tartrate preparation: The basic method is carried out through step 5. The sections are then transferred to the following mixture: To 40 cc. of Steiner's double tartrate–silver nitrate solution, contained in a Coplin jar, 10 cc. of a 5 per cent solution of rosin in alcohol is added rapidly. The sections are treated with the resulting milky fluid for one hour at room temperature. The basic process is then continued, beginning with step 7.

2. Ox bile: This variation is of particular interest, since sensitization of the impregnated section is accomplished by the selective action of a normal organic secretion without the need of a silver salt. The basic method is carried out through step 4. The sections are then transferred to a sensitizing solution consisting of

40 cc. of a 10 per cent dilution of fresh ox bile in water and 10 cc. of a 5 per cent solution of rosin in alcohol. The sections are treated for one hour at room temperature in the resultant yellowish milky fluid, and then the routine process is continued, commencing at step 7.

Substitutes for Rosin in the Sensitizer.—1. Gum mastic: The basic method is followed through step 5. The sections are then treated for one hour at room temperature with the following milky preparation: To 40 cc. of the silver nitrate–lithium carbonate solution 10 cc. of a 2.5 per cent solution of gum mastic in alcohol is added, rapidly. The flask is shaken gently. Steiner's silver tartrate solution can be used in place of the silver-lithium solution. After this treatment of the sections the routine process is resumed, beginning with step 7.

2. Ox bile: The basic method is followed through step 5. The sections are then sensitized for one hour at room temperature in a greenish yellow and opalescent mixture of 45 cc. of silver nitrate–lithium carbonate solution and 5 cc. of fresh ox bile. The bile is added to the silver–lithium solution and mixed by shaking. If desired, Steiner's silver tartrate preparation can be used in place of the silver–lithium solution. After this treatment the routine process is resumed from step 7.

3. Balsam of Tolu: The basic method is followed through step 5. The sections are then sensitized for one hour at room temperature in a milky mixture of 45 cc. of silver nitrate-lithium carbonated solution and 5 cc. of a 1 per cent solution of balsam of Tolu in alcohol. Following this treatment the routine process is resumed from step 7 on.

Substitute for Solution of Formaldehyde–Pyrogallic Acid Reducing Agent.—Hydroquinone: A solution of 1 Gm. of hydroquinone in 60 cc. of distilled water can be substituted for the formaldehyde-pyrogallic acid reducing agent. The sections are treated for ten to fifteen minutes at room temperature.

RAPID METHOD

Solutions Required.—1. Silver nitrate: A 1 per cent solution in water. Preferably this should be prepared just before using.

2. Lithium carbonate: A dilution is made consisting of 1 cc. of a cold saturated aqueous solution (about 1.3 per cent) in 165 cc. of distilled water. This diluted solution will keep for about five days at room temperature. If lithium carbonate is not available a 0.1 per cent solution of sodium and potassium tartrate may be used with equally satisfactory results.

3. Rosin: A 2 per cent solution of rosin in alcohol is used. This is made as follows: A 50 per cent solution of rosin in absolute alcohol is prepared. After the rosin is completely dissolved, it is passed three times through filter paper. It may be stored in the refrigerator for long periods. Just before use it is diluted twenty-five times with alcohol to a final concentration of 2 per cent.

4. Levaditi's reducing solution: This is the same as that used for the "basic method."

Procedure.—1. Paraffin-embedded sections, cut 4 to 6 microns thick, are fixed on slides; the paraffin is removed, and the sections are hydrated in the usual manner as far as distilled water.

2. The sections are transferred to the following reagent, prepared just before use: silver nitrate solution, 30 cc.; lithium carbonate solution, 10 cc.; rosin solution, 10 cc. The lithium carbonate and silver nitrate solutions are first mixed in a Coplin jar. To this opalescent mixture the rosin solution is added rapidly, and the mixture

is well shaken. The sections are treated in this milky preparation for one hour at 56 C.

3. The sections are transferred directly to a reducing mixture with the following composition: Levaditi's reducing solution, 50 cc. rosin solution, 5 cc. The sections are treated in this preparation for seven to ten minutes at room temperature.

4. Wash in two or three baths of 90 per cent alcohol for five minutes each.

5. Wash rapidly in distilled water.

6. Replace in the silver–lithium–rosin solution used in step 2 for an additional fifteen minutes at 56 C.

7. Transfer directly to the reducing solution used in step 3 for a second treatment of three to five minutes at room temperature.

8. Wash in two 90 per cent alcohol baths, two minutes in each bath.

9. Dehydrate and mount in Canada balsam.

In case unsatisfactory results are obtained the corrective procedure described for the basic process may be applied.

COMBINED PROCESS FOR DEMONSTRATING SPIROCHETES AND PATHOLOGIC CHANGES

Frequently it is desirable to study the pathologic changes in the tissue preparations as well as to demonstrate the presence of spirochetes. For this purpose the "rapid process" can be used with two variations: (1) the omission of rosin in the reducing solution and (2) the subsequent use of a contrast stain. In detail the process is as follows:

1. The rapid process is carried out through step 8 with the exception that the reducing solution used in steps 3 and 7 contains no rosin.

9. Wash in distilled water.

10. Stain the sections for ten to fifteen minutes in Harris' hematoxylin.

11. Pass rapidly through the diluted lithium carbonate solution.

12. Wash in running water.

13. Stain one to three minutes in Altmann's acid fuchsin which has been diluted 1:5 in distilled water just before using.

14. Differentiate in absolute alcohol for five minutes.

15. Dehydrate and mount in Canada balsam.

By this process the cytoplasm is stained light red, the nuclei dark violet, the connective tissue bright red or ochre yellow and the spirochetes black.

Satisfactory results can also be obtained with other counterstains as follows:

Van Gieson's stain: This is used in place of diluted Altmann's fuchsin. The staining time is one minute.

Meyrick and Harrison stain: This stain was orginally recommended by Meyrick and Harrison[6] as a background stain for their application of Gram's method to paraffin-embedded tissues. It is composed of 15 parts of an aqueous 1 per cent solution of neutral red and 1 part of carbolfuchsin (a mixture of 9 cc. of an alcoholic 10 per cent solution of basic fuchsin and 90 cc. of 5 per cent phenol in water). The mixture remains unchanged for long periods. It is used as follows:

6. Meyrick, L. D., and Harrison, C. V.: J. Path. & Bact. **54**:517, 1942.

1. The rapid process is followed through step 8 except that the reducing solution used in steps 3 and 7 has no rosin.
9. Wash in distilled water.
10. Stain for ten minutes.
11. Differentiate rapidly in absolute alcohol.
12. Dehydrate and mount in Canada balsam.

By this process the cytoplasm is stained light red, the nuclei bright red, the connective tissue dark red or greenish yellow and the spirochetes black.

COMMENT

The accurate and rapid demonstration of spirochetes in tissue sections is of distinct histologic value. Not only can lesions associated with the presence of these organisms be studied, but, in addition, the demonstration of the organisms simplifies recognition of various visceral lesions whose polymorphous nature would not otherwise permit a definite pathologic diagnosis. The value of such a technic is enhanced when the histopathologic material available for examination is limited. Such is often the case with viscerotomy specimens.

The use of the method described is practical, since the necessary reagents are inexpensive, easily obtained and commonly used in histologic technic.

The new method of silver impregnation, based on the use of a colloidal suspension of lithium carbonate and silver nitrate, can be utilized in various ways, the principal modification being the "rapid method." The latter technic, which dispenses with a mordant and reduces the essential procedure to a double process of impregnation and reduction in a colloidal medium, can be performed in two hours. In addition, aniline counterstains can be used.

Other variations of the basic method are described for use when chemicals necessary for the standard method are not available. These alternatives are listed in the order of preference.

It is worth while to emphasize the possibility of using ox bile in the silver impregnation of spirochetes in tissue sections, since this has been found to be an excellent substitute either for the rosin or for the lithium-silver complex. Of all reagents used, bile is the only one in which this dual capacity has been demonstrated. Furthermore, it is easy to obtain.

I have observed comparable and regular results with both the basic and the rapid method, not only when these results were checked against those given by the original Levaditi method for tissue blocks in solution of formaldehyde, but also when they were checked against those given by the Steiner method. For this study I used viscerotomy, autopsy and experimental material, containing Treponema pallidum, Treponema pertenue or Leptospira icterohaemorragiae.

From a practical point of view either of the two methods described can be used. However, if there is no great urgency in arriving at a diagnosis, or if there is only a small amount of material available it is wise to use the basic method, carried out at 37 C., which gives certain and uniform results with any type of tissue.

SUMMARY

A new method for silver impregnation of spirochetes in paraffin tissue sections mounted on slides provides accurate and rapid demonstration of these organisms. There are two variations of the method, the "basic method," whose fundamental reagents are uranium nitrate, silver nitrate, lithium carbonate, rosin and Levaditi's reducing solution, and a "rapid method," in which a double process of impregnation and reduction avoids the use of a mordant. The fundamental principle involved in both technics is the use of a lithium-silver complex in a colloidal medium to obtain selective impregnation of the spirochetes.

Possible variations in the technic give a wider range of application and practicability.

Books Received

HUMAN EMBRYOLOGY. By Bradley M. Patten, professor of anatomy in the University of Michigan Medical School. Pp. 776, with 1,366 drawings and photographs grouped as 466 illustrations, 53 in color. Price $7. Philadelphia and Toronto: The Blakiston Company, 1946.

The book is planned in such a way that each subject is handled in a stimulating manner to emphasize its importance in the practice of medicine. Developmental processes are presented as a succession of dynamic phases in a continuum, not as widely separated steps. Primordial stages in the morphogenesis of the embryo, the mechanism of vesicular implantation in the uterus, and the concomitant changes in the reproductive organs of the mother, which are so important in gynecology and obstetrics, have received particularly careful attention. Among the other features emphasized are the following: the advanced stages in organogenesis, basic to an understanding of the plan of the body as demonstrated in gross anatomy; the histogenesis of some of the major organs, calculated to explain the architecture of viscera as encountered in microscopic anatomy; the more common developmental anomalies, described because of their clinical importance. A special chapter is included to facilitate correlation of text and laboratory study. The contents of the volume record its broad sweep: reproductive organs; gametogenesis; the sexual cycle and fertilization; cleavage, formation of germ layers and establishment of the embryonic body; early differentiation of the body and establishing of organ systems; fetal membranes and placenta; age, growth and changes in external form of the body; twinning, double monsters and teratology; integumentary system; connective tissues and skeletal system; muscular system; development of the nervous system; organs of special sense; development of the face, jaws and teeth; development of the digestive and respiratory systems; body cavities and mesenteries; ductless glands and pharyngeal derivatives; development of the urogenital system; development of the circulatory system. Forty-two pages are devoted to a bibliography which selectively lists the major sources of the information discussed. There are 1,366 drawings and photographs, grouped as 466 illustrations. Many of the illustrations are original drawings by the author; of the total number, 53 are in color. The textbook contains also serviceable graphs and semidiagrammatic figures illustrating skeletal, visceral and general body growth, the bases of anomalous development of organs and of persistence of vestigial structures. The text is readable; it flows with a facility which has pleased all readers of Professor Patten's scholarly manuals and journal articles. While "Human Embryology" is a new book, it carries every sign of maturity; it is the outgrowth of rich experience in teaching and in research achievement.

DR. F. G. GADES PATHOLOGISK-ANATOMISKE LABORATORIUM I BERGEN. Meddelelser 1940-1941 and 1942-1945. Various pagination. 1942 and 1946.

NOUVELLES ÉTUDES CLINIQUES ET BIOLOGIQUES SUR LA PATHOLOGIE DU FOIE. By Étienne Chabrol, professeur de Clinique Médicale à la Faculté de Paris. Membre de l'Académie de Médecine. Paper. Pp. 182, with 24 figures. Paris: Masson et Cie., 120 Boulevard Saint-Germain, 1946.

This monograph is composed of twenty-one lectures on selected topics in disease of the liver. The first three chapters constitute an interesting historical introduction to the contributions of the French in the early descriptions of hepatic disease. The remaining discussions center about case presentations. Two lectures touch on epidemic hepatitis, but the author appears unaware of the important recent

contributions from other countries. The illustrations give little information. The book is inaccurately titled in view of the fact that it is chiefly a review of previous publications of the author and his colleagues.

QUANTITATIVE CLINICAL CHEMISTRY: INTERPRETATIONS. By John P. Peters, M.D., M.A., professor of internal medicine, Yale University School of Medicine, and Donald D. Van Slyke, Ph.D., Sc.D., member of the Rockefeller Institute for Medical Research. Volume I. Second edition. Pp. 1041, with 62 illustrations. Price $7. Baltimore: Williams & Wilkins Company, 1946.

The long-awaited second edition of this monumental handbook of clinical chemistry has finally arrived. At least part of it has, for the material covered is so vast that only the sections on total metabolism, carbohydrate, lipids and proteins could be included in this first volume of interpretations. The remaining subjects will be included in a later second volume, whereas methods will constitute a third. As it is, this small portion comprises 1,041 pages, compared with 1,264 of the complete first edition. It is principally written by Peters.

The subject of carbohydrates, which in the first edition was covered in probably the least authoritative chapter, is in this edition brilliantly expounded. The discussion of the biochemistry of intermediate carbohydrate metabolism, the role of insulin and other hormones and the vicissitudes of the blood glucose concentration and the clinical story of diabetes demonstrate a mature, broad-minded blending of the sciences of biochemistry, physiology, endocrinology, nutrition and clinical medicine. The assisting hand of the trained biochemist and carbohydrate authority C. N. H. Long is visible throughout the whole section.

The chapter on lipids has a similar wide scope, but the still uncrystallized state of the interpretation of the clinical variation of the individual blood lipids is disappointing; for one always imagines that authors of a monograph can bring order out of the chaos of the many controversial and mutually contradictory physiologic and clinical studies. Also, one hopes in vain that the survey of the 978 papers on which the exposition of lipids is based can lead to a clear picture of the metabolism of lipids in hepatic and other diseases. But the fault, of course, is not with the authors.

The steroid hormones are discussed all too briefly in 12 pages.

Part IV covers the subject of the metabolism of nitrogen in 6 chapters. Along with the retained description of plasma proteins and urea and the clinical story of Bright's disease—which were among the outstanding features of the first edition—is offered an up-to-date and authoritative elucidation of the role of protein nutrition in disease processes. Here the broad experience of both authors is easily recognizable. The chapter on purines has relatively little new material except for the isotopic studies of Schoenheimer.

There is little doubt that the book will be received with as much acclaim as the first edition, and deservedly so. Yet in spite of the broad biochemical and clinical experience of the authors and their indefatigable compilation, the limitations of a book with such ever-widening scope written by single authors is evident from the fact that there are few references after 1943.

HÉPATITES RARES. By Maurice Loeper, professeur de Clinique Médicale à la Faculté de Médecine de Paris, et membre de l'Académie de Médecine. Paper. Pp. 214, with 17 figures. Price 290 francs. Paris: Masson et Cie., 1946.

In this monograph rare diseases of the liver are discussed in 21 chapters, together with some complications of hepatic disease and certain aspects of hepatic function. The illustrations are poor, and the bibliographies are brief. The book is well written, interesting, informative and at times provocative.

News and Notes

Appointments, etc.—R. S. Spray, for twenty-five years a member of the faculty of the West Virginia University School of Medicine, Morgantown, has retired as professor of bacteriology.

H. P. Rusch, professor of oncology in the University of Wisconsin, has been appointed director of the McArdle Memorial Laboratory for Cancer Research of that university.

J. G. Hoffman, Ph.D. in physics, has been appointed director of cancer research in the Rockwell Park Memorial Institute, Buffalo.

The retirement of Colonel James E. Ash, M.C., director of the Army Institute of Pathology, Washington, D. C., has been announced.

H. M. Weaver, Wayne University College of Medicine, Detroit, has been appointed director of research of the National Foundation for Infantile Paralysis.

E. L. Miloslavich, professor of pathology in Marquette University Medical School, Milwaukee, from 1920 to 1933 and professor of legal medicine in the University of Zagreb, Yugoslavia, from 1933 to 1944, has returned to this country.

H. S. Breyfogle, director of the medicolegal department of St. Louis County, in Missouri, has been appointed chief medical examiner for the State of Virginia under the provisions of the act of the legislature establishing a new system of examinations and reports of deaths occurring under unnatural or suspicious circumstances.

Deaths.—Sophia Getzowa, professor emeritus of pathology at the Hebrew University in Jerusalem, died July 12, 1946, at the age of 74. She was the pioneer pathologist in Palestine, making autopsies in all parts of the country and pathologic examinations for all the hospitals for many years.

Winford P. Larson, head of the department of bacteriology of the University of Minnesota since 1918, died January 1 at the age of 66.

Eugene C. Piette, pathologist of the West Suburban Hospital, Oak Park, Ill., died at the age of 54. He was formerly associate professor of pathology in the University of Illinois and for a short time (1918-1920) professor in the University of Kharkov, Ukranian S. S. R., Soviet Union.

Society News.—The Society of American Bacteriologists will hold its forty-seventh annual meeting in Philadelphia, May 12 to 16, 1947.

The American Society of Clinical Pathologists will hold its twenty-sixth annual meeting in Atlantic City, N. J., June 6 to 8, 1947. The headquarters will be the Ambassador Hotel.

The Memphis Society of Pathologists has been formed, with D. H. Sprunt as chairman.

The Pennsylvania Association of Clinical Pathologists has been organized. William P. Belk, Philadelphia, is the president, and H. F. Hunt, Danville, is secretary-treasurer.

Hortega Memorial.—*Archivos de histologia normal y patologia* dedicates its June 1946 number to the memory of its founder, Pio del Río Hortega, neurohistologist, 1882-1945. The number contains notable articles on neurobiologic problems.

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